

Tag (see P29)

Project No. \_\_\_\_\_ Appl. No. 09/006,421

Book No. \_\_\_\_\_

65

age No. \_\_\_\_\_

Tag assay mix (P557)  
except no activated DNA

[A]

(40 Rxns)

0.5 M Tris pH 8.3

100

✓

1 M MgCl<sub>2</sub>

4

✓

3 M KCl

33.3  $\mu$ l

✓

1 ATGC-TP 10  $\mu$ l each

40

✓

2 <sup>32</sup>P dCTP

5.1 4.2  $\mu$ l

✓

H<sub>2</sub>O

10.2 19  $\mu$ l

✓

(1) 385  $\mu$ l  $\rightarrow$  1.4  $\mu$ l  $\rightarrow$  385

(2) (use 35  $\mu$ l / 5  $\mu$ l rxn)

(same as P17)

3  $\mu$ l 19 500 pmoles /  $\mu$ l

13.3  $\mu$ l

66.7  $\mu$ l

e. 11 Rxns

0.165  $\mu$ g /  $\mu$ l

H<sub>2</sub>O

96.7

48.5

(45  $\mu$ l / 5  $\mu$ l)

① = 0.2  $\mu$ g DNA / 5  $\mu$ l

② = 1  $\mu$ g DNA / 5  $\mu$ l

(= 5  $\mu$ l rxn / 5  $\mu$ l)

48.5

48.5

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

45  $\mu$ l  $\rightarrow$

40  $\mu$ l  $\rightarrow$

total units

CKBTI

78

5

5

0.4

6

5

5

0.8

25

5

5

1.5

5

5

5

3.125

5

5

6.25

5

5

12.5

5

5

25

5

5

50

5

5

100

5

5

200

7  $\mu$ l

50  $\mu$ l

2 min at 74°C  $\rightarrow$  add 1  $\mu$ l EDTA  
spot 40  $\mu$ l on GFC

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Used & Understood by me,

Enrica Polansky

Date

10/24/94

Invented by

Recorded by

Date

10-18-94

Sm Page No. \_\_\_\_\_

	units / 50 $\mu$ l			pmol
0.2 $\mu$ g DNA	0.4	1	133.00	2.6
	0.8	2	248.00	4.9
	1.6	3	264.00	5.2
	3	4	470.00	9.2
	6	5	633.00	12.5
	12	6	886.00	17
	25	7	991.00	19.5
	50	8	995.00	18.6
	100	9	999.00	18.7
	200	10	883.00	17.4
1.0 $\mu$ g DNA	0.4	11	2146.00	42
	0.8	12	3847.00	73
	1.6	13	6695.00	133
	3	14	12077.00	238
	6	15	17179.00	339
	12	16	17333.00	342
	25	17	22279.00	440
	50	18	22941.00	452
	100	19	23863.00	471
	200	20	24510.00	477
	500	21	92.00	
	1000	22	304197.00	
②				76 cpm/pmol

76 cpm/pmol

need time course at high and low [Tag]  
to see if lag plays a role. In a PCR with  
15-30 min elongation time, effect of lag would  
be minimized

Results per Tag spot =  $100,000$  u/mg  
25 units = 50 nm Tag  
50  $\mu$ l

Both plots (0.2 and 1.0  $\mu$ g DNA) saturate 50 nm Tag  
suggesting an equilibrium effect of pol DNA binding,  
rather than titration of pol at 1 pol/mg DNA  
1  $\mu$ g DNA, saturation at 10 n / 0.42 pmol circles

for 200,000 u/mg Tag

1 unit Tag = 0.053 pmol molecules  $\sim$  1:1 Tag/circle

1  $\mu$ g mp19  $\Rightarrow$   $\frac{1 \times 10^{-6} \text{ g}}{(330 \text{ g mole}^{-1})(7250 \text{ bp})} = 0.42 \text{ pmol circle}$

Project No. \_\_\_\_\_  
Book No. \_\_\_\_\_

TITLE Tag - Mutant  $\rightarrow$  Heparin Pool over  
Super Q GSD

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so Heparin Pool  $\rightarrow$  dialyzed against Bfr A  $\rightarrow$  250 mL - 2 exch  
~ 12 mL -

Bump 5 mL Super Q GSD column w/ Ga HCl + NaCl  $\rightarrow$

Wash w/ H<sub>2</sub>O

Equilibrate w/ Bfr A inlet conductivity 1.37 mS  
outlet conductivity 1.42 mS

Sample - 1.5 mS

saved 1 mL of Load material - Load ~ 11.5 mL -  
collect Load flow through + wash

Wash w/ Bfr A - Flow rate - 1 mL/min -

Gradient Bfr A  $\rightarrow$  Bfr B  $\rightarrow$  10 vts - 50 mL total  
collect 1 mL fractions -

Pool 10-12 dialyze against storage buffer -

Sign premix - add 11  $\mu$ L hot dCTP -

5  $\mu$ L / rxn - .5, 1, 2, 4  $\mu$ L - enzyme dilute 1/20

DS 1	62892.00	900
1 2	53562.00	
1/2 3	80556.00	Q Pool
2 4	80834.00	
1/2 5	39642.00	Q Pool
1/2 6	55734.00	
2 7	61384.00	
4 8	69380.00	1/1
1 9	49764.00	
1/2 10	42686.00	Hep Pool -
4 11	75336.00	Q load
1 12	60344.00	
1/2 13	50018.00	Hep load -
4 14	57888.00	
15	652.00	

1	1	7260.00	
1/3000 2	2	7498.00	129 $\mu$ /mL
3	4	13534.00	1174 $\mu$ /mL
1/2000 4	1	2836.00	
5	2	4118.00	
6	4	4440.00	

SA = 78 cpm/pmol

Factor =  $1.154 \times 10^{-5}$

To Page 1

Witnessed & Understood by me,

Date

Invented by

Dat

Record d by

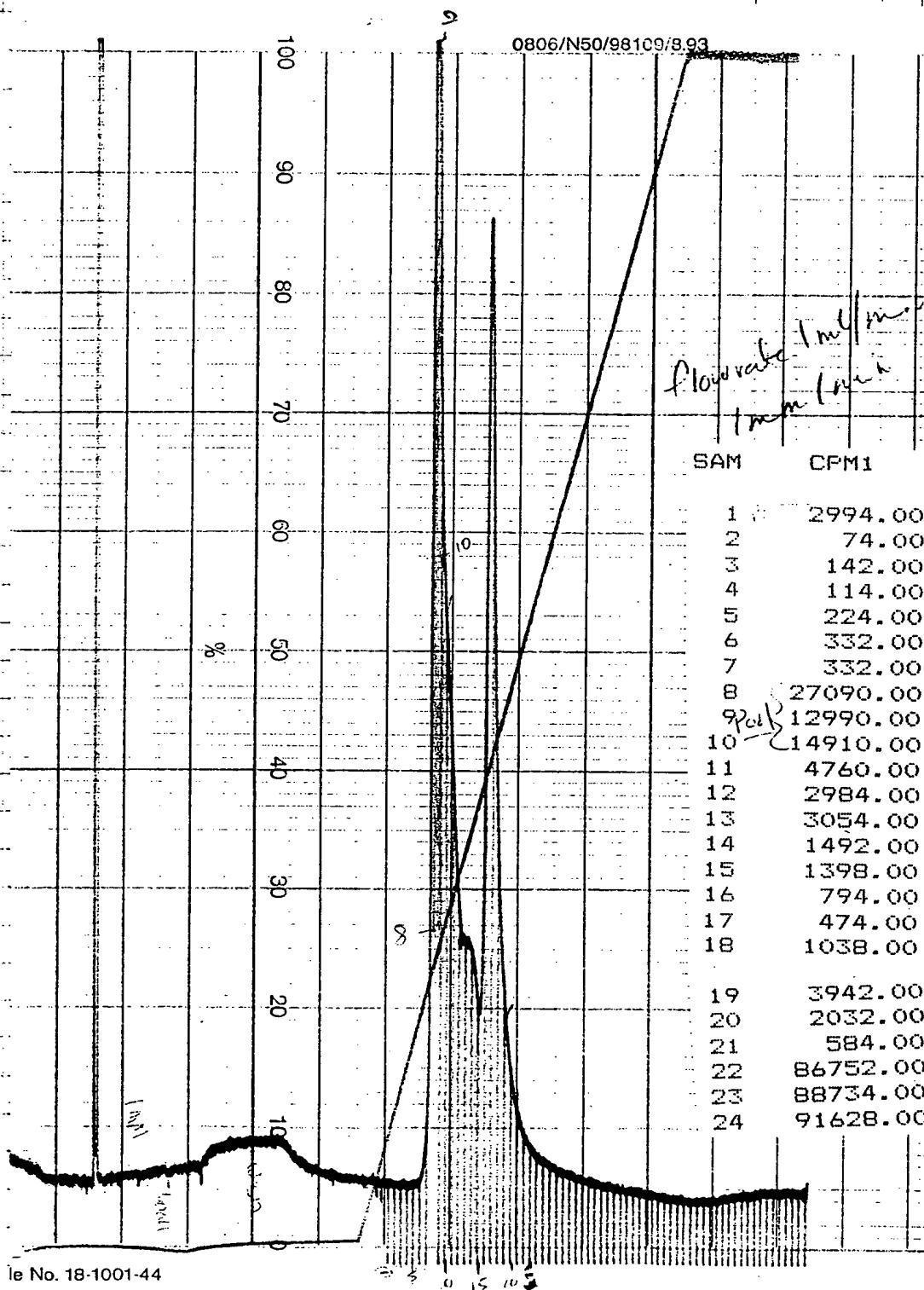
Super Q-650-

Pr j ct No. \_\_\_\_\_

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25 ml H<sub>2</sub>O  
+  
1.5 ml sample  
↓  
74°C - 7 min.

ice - 10% S.M.S.M.  
spin  
↓  
spot 20 µl GF10  
TLC wash.

- ① LM-410
  - ② PT
  - ③ WFT
  - ④ S
  - ⑤ 6.7
  - ⑥ 8
  - ⑦ 9
  - ⑧ 10
  - ⑨ 11
  - ⑩ 12
  - ⑪ 13
  - ⑫ 14
  - ⑬ 15
  - ⑭ 16
  - ⑮ 17
  - ⑯ 18
  - ⑰ 19
  - ⑱ 20
  - ⑲ 21
  - ⑳ 23
  - ㉑ 21
  - ㉒ 22 → 2 µl mix
  - ㉓ 25
- 1/10 dilution

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Used & Understood by me,

jk

Date

2/21/95

Invented by

C. Lynn

Recorded by

Date

12/94

T. neapolitana DLE (2779) SOM

g No. 48

October 13, 1994 (Thursday)

I infected DH12S cells with the  $\phi$  from #5, #6, #7 (2m cells  
 grown in 2x YT + 5  $\mu$ l  $\phi$ ; grown 37°C (air shaker) 16 hrs)

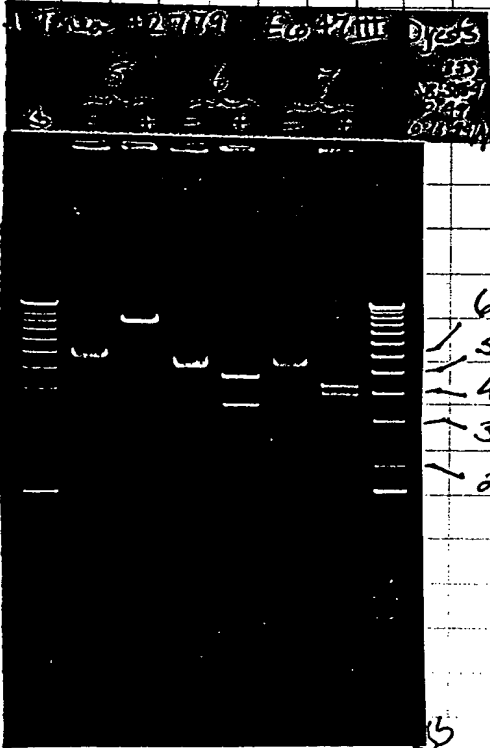
RF isolated by alkaline/SDS except 1  $\mu$ l RNase A (1mg/ml) added  
 to prep at 10Hq OAc addition

RNA dissolved in 50  $\mu$ l T<sub>10</sub>E<sub>1</sub>

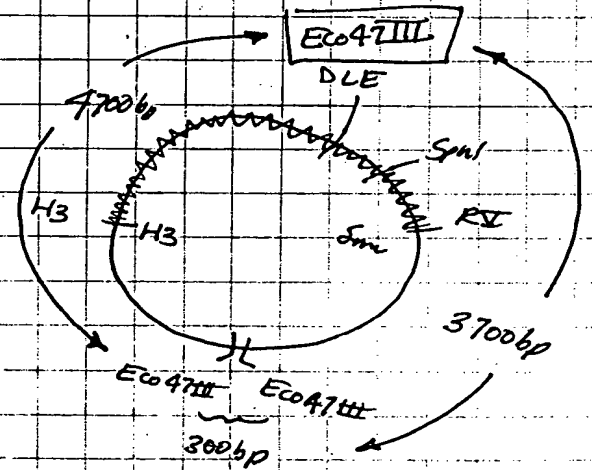
### DIGEST SCHEME

(React 3)	HOP	10 $\mu$ l	✓	Incubated 37°C (heat block) 1 hour.
	10x B/R	2	✓	
	DNA	7	✓	
(4 $\mu$ l)	Eco47III	1	✓	
	Total	20 $\mu$ l		

3% Agarose Gel (1xTAE); 190V/16



Comments:



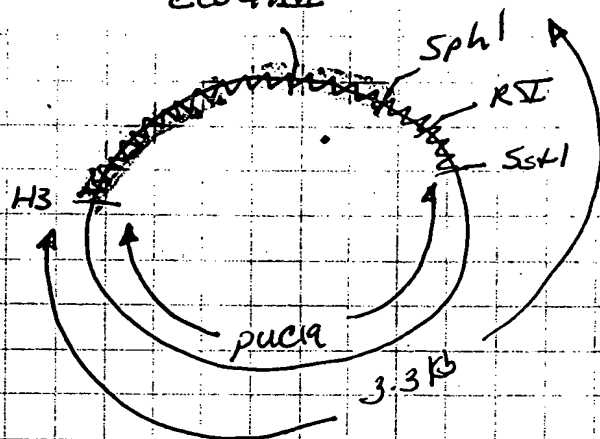
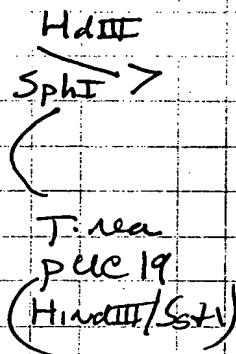
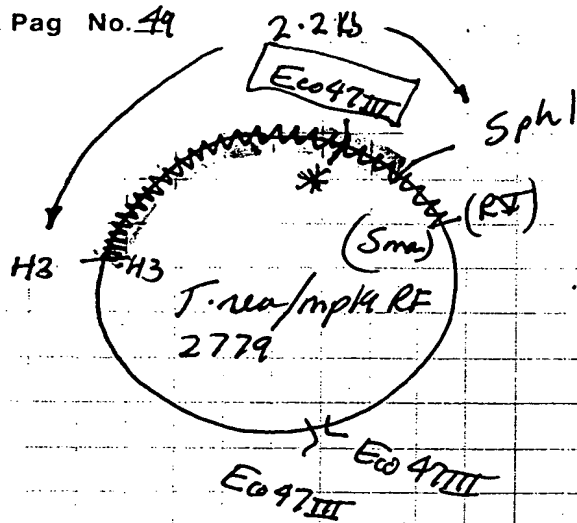
The bands are migrating  
 at the expected distances for  
 #6. There must have been  
 overabundance of some  
 "component" causing the DNA  
 to run faster. I will clone  
 into the pUC vector.

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Read & Understood by me,	Date	Invented by	Date
May 10/94	10/24/94	Recorded by	10-13-94

October 13, 1994 (Thurs)

EC 4711



## DIGEST SCHEME

DIGEST SCHEME			#6
		<u>T. nuc / pUC</u>	<u>2729 / mp19</u>
(React 2)	HOM	12 $\mu$ l ✓	6 $\mu$ l ✓
	10x Bfr	2 ✓	2 ✓
	DNA	4 ✓	12 ✓
(104 $\mu$ l)	HindIII	1 ✓	1 ✓
(104 $\mu$ l)	SphI	1 ✓	1 ✓
	Term	20 $\mu$ l	20 $\mu$ l

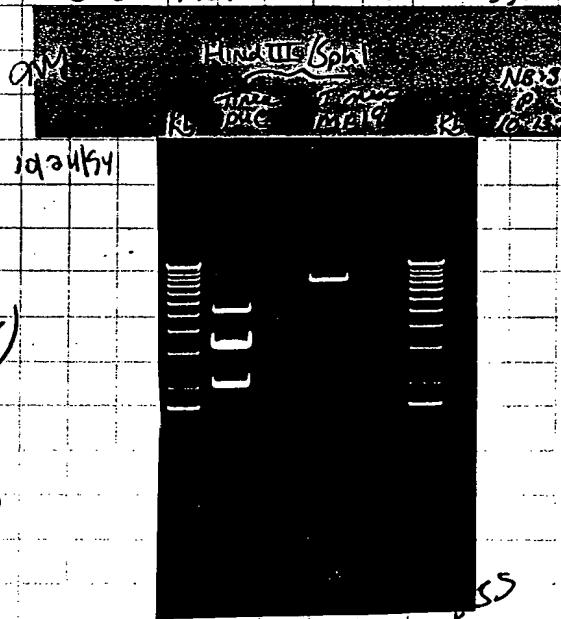
Incubated 37°C (hot start)  
1:10 → 1:50

0.8% Agarose Gel (XTAB); 1%  
 100bp DNA Ladder

Comment: I should see

a. 3.3 Kb (desired fragment)

and a 2.2 Kb fragment from the T<sub>ne</sub>/pUC19 clone and I do. Unfortunately I should see a 7.9 Kb fragment and 2.2 Kb (deleted fragment) from the T<sub>ne</sub>/mp19 (2779 ~~66~~6) RF DNA and I don't. Both sites were present before I performed the mutagenesis (see p. 35) - I will have to repeat.



To Page No

**Witnessed & Understood by me,**

**Date****Invented by**

**Dat**

May Jones

10/24/94

**Recorded by**

10-13-94

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE Deep Vent / GAPDH / diff primers

16

11/16/94

Form Page No. \_\_\_\_\_

Purpose: Since GAPDH - PCR worked with 3' Thiol primers attempted the same amplification with other available primers, under same conditions.

- Deepvent buffer enzyme at 1U and 0.5U
- 200 pg Template Mg at 2, 3, 4 and 6 mM
- 200  $\mu$ M dNTP
- 1  $\mu$ M primers

- did just one of each. primers
- \* 2697 & 2696 no do Lac FWD & Lac Reverse (100  $\mu$ M)
- + dU " " 3'-1 PPT
- (10  $\mu$ M)

- each primer set - 10x RX were made.

	Regular	17-20 = 0.5 U	dU	23-36 = 0.5 U	3'-1 PPT	
		21-24 = 1.0 U		37-40 = 1.0 U		25
10x buffer	50		50		50	29
dNTP	10		10		10	
primer 1	5		50		20	
2	5		50		20	
Template	20 (100 pg/x)		20		20	
H <sub>2</sub> O	260		270		330	

450  $\rightarrow$  45  $\mu$ L/RX  $\leftarrow$  450  $\leftarrow$  450

added 5 $\mu$ L of	2	3	4	6	mM	67-20
Mg chlorination	1	1	1	1		
	0	0.5	1	2	(100 mM)	10x
	5	4.5	4	3	1120	

- enzyme added individually 0.25  $\mu$ L for 0.5 U
- 0.5  $\mu$ L for 1 U

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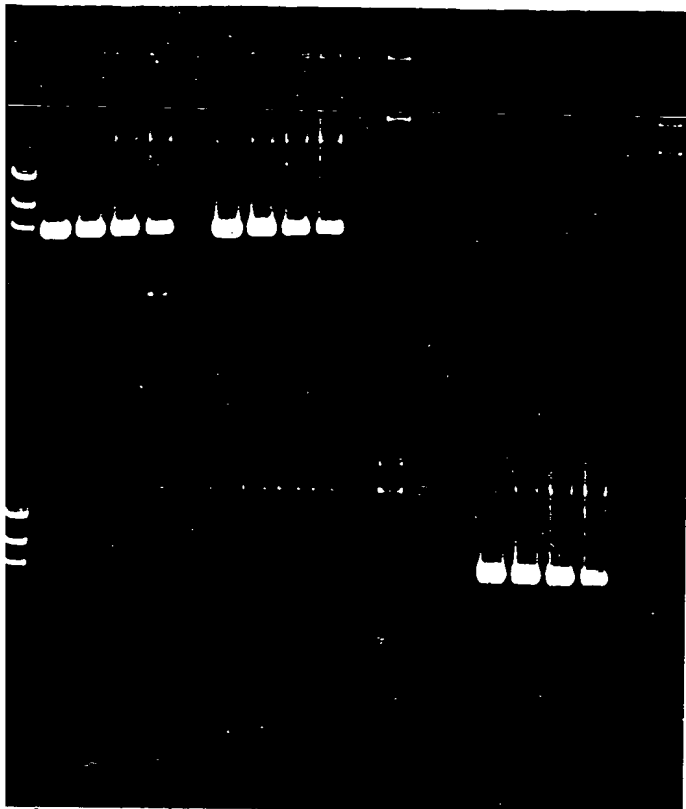
K. Subramani

11/16/94

g N Regular

3'-1 PPT

0.5V

2 3 4 6  
it 0.52 3 4 6 mol 12g  
1V

du primer

Vent +/- exo

didn't discriminate

between modified and unmodified

With du in earlier lines per 2 500 bp lac 2 never got amplified

- samples divided

12/19/94

Result

- Regular - unmodified, revised, so couldn't say 3'-1 PPT is better than unmodified!

- du certainly has problem with Deep Vent.

- with the amount of product seen with 1V don't know why there is no product with 0.5V & 3'-1 PPT primers.

Can of template - ?  
Centaminatin -  
what are these bands on the top.

- Template / primer - no enzyme controls also have them?

To Page No. \_\_\_\_\_

ed &amp; Understood by m ,

Date

11/28/94

Invented by

Recorded by

K. Stachman

Date

11/18/94

# UNIT ASSAY FOR T. mea Pol.

ag No. 2/1/95 Purpose: Determine the unit concentration of two samples.

## Premix:

✓ 335  $\mu$ l 2x Reaction Buffer (150 mM TAPS, pH 9.3 / 50 mM KCl / 1 mM DTT)  
✓ 1.3  $\mu$ l  $HgCl_2$  @ 1.00 M  
✓ 92.9  $\mu$ l DHA @ 3.5  $\mu$ g/ $\mu$ l  
✓ 139.8  $\mu$ l  $H_2O$   
✓ 65  $\mu$ l H.C. @ 90.3 cpm/pmol

## Reaction Buffer:

I. 0.5 M Taps, pH 9.3 180 ml  
FW = 243.3 g/mol 12.165 g  
Titrated with 10 N NaOH

II. For 10 ml @ 2x

✓ 1 ml 0.5 M Taps, pH 9.3 = 50 mM  
✓ 500  $\mu$ l 2 M KCl = 100 mM  
✓ 40  $\mu$ l 0.5 M DTT = 2 mM

Assay @ 72°C, 10 min

	156	U/ml
1 $\mu$ l of 53 $\mu$ l @ Yeo	65413	17.3
2 $\mu$ l	113154	15.0
3 $\mu$ l	151375	13.4
1 $\mu$ l of 5 $\mu$ l @ Ys	44554	1.48
2 $\mu$ l	95434	1.58
3 $\mu$ l	121699	1.35
1 $\mu$ l JH-61 @ Yeo @ Y100	19038	

$\bar{X} = 15.2$  U/ml  
 $\bar{X} = 1.47$  U/ml

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Assessed & Understood by me,

R. Plen

Date

7/12/95

Invented by

Recorded by

Date

5/23/95

TNE

Page No. \_\_\_\_\_

12/95

Goal: To clone the TNE 35FY (mut) into pUC99A.

pUC TNE 35FY clone #1	30	pUC99A	5
10x R4	5	10x R2	2
H <sub>2</sub> O	13	H <sub>2</sub> O	11
BspHI	2	NcoI	1
	50 $\mu$ l	H3	1

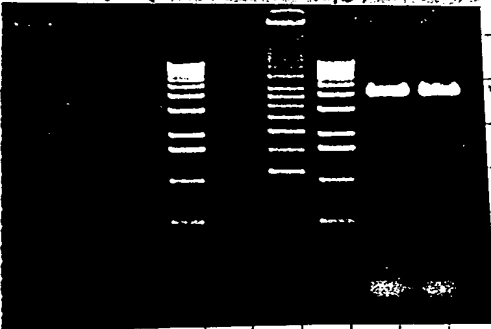
37°C - 1 hr.

Applied 5  $\mu$ l to 0.8% agarose gel run at 180V

20  $\mu$ l

Applied to 0.8% agarose gel run at 180V.

Sl. uncut pUC99A 35FY #1  
pUC99A 35FY #1  
BspHI



mut

cut out frag & freeze at 20°C

pUC99A/NcoI/H3 cut looks good  
cut out 56 bp  
4176  
- 56  
4120 bp

pUC TNE 35FY #1 / BspHI gives 1 kb, 1.3 kb, + 2.7 kb frag. Therefore, BspHI cuts pUC TNE 35FY #1 3X. There must be a BspHI in the insert.

13/95

ETOH ppt. Digest.  
Dissolved in 20  $\mu$ l TE

BspHI

BspHI

5  $\mu$ l  
5  $\mu$ l 1X BT  
10  $\mu$ l

15  $\mu$ l DNA  
2  $\mu$ l 1X R2  
2  $\mu$ l H<sub>2</sub>O  
1  $\mu$ l H3 10  $\mu$ l  
20  $\mu$ l

Applied to 1 lane of 0.8% agarose gel. Gel run at 180V

37°C - 1 hr.

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Used & Understood by me,

Date

Invented by

Date

Lisha Xu

7/14/95

Recorded by

My Long

7/13/95

From Page No. \_\_\_\_\_

- Take pool from Hep. (cancer) and load into Q (cancer) syringe

BFR A.

(12)

2.5 mm Tris - 7.4 2.5 ml  
 0.5 mm EDTA 1 ml  
 5 mm Bml 3.58 ml  
 10% glycerol 10 ml  
 10 mM KCl 3.3 ml  
 95-100 dH<sub>2</sub>O (ms = 1.9)

- Take Bml from pool -

is in 5. BFR 2.5 ml 1:5 w/ 500 ml 3500 ml A  
 load. at 1 ml/min. (1.7 ms)

B = same (+) 1 M KCl. (6.7 ms)

1 - SA

2 - Bkg

3 - C

4 - CFA

5 - 10

6 - 15

7 - 18

8 - 20

9 - 22

10 - 24

11 - 24

12 - 28

13 - 30

14 - 32

15 - 34

61656.00

62.00

974.00

76.00

48.00

84.00

3696.00

1112.00

762.00

554.00

454.00

330.00

120.00

150.00

118.00

low SA - need new viral

1. 2. 3. 4. 5. 6. 7. 8. 9. 10. 11. 12. 13. 14. 15.

0.5 ml sayle - in 7.4 C / 10 ml

Total units

% Known

Blue load 3550

Blue pool 1500

- (42%)

Hep load 3550

Hep pool 5470

+ (154%)

Q load 1248

Q pool 560

(45%)

1514.00

2178.00

3058.00

2722.00

4326.00

4726.00

125066.00

125278.00

Test for Activity at 607 Fy (F607Y). Check for pol. activity post 1000 kills.

- Disease per per 80 pools

from Del's clone per 124, 3000 3573, 41 + color

- Spec Act = 78.3

- Coolers good! Activity post let need Del's clone for per

Witnessed &amp; Understood by me,

Date

Invented by

Date

Recorded by

To Page N

<sup>32</sup>P primer for 14/ Vent  
Human spleen DNA

Project N \_\_\_\_\_

Exhibit 2

Appl. No. 09/558,421

B k N \_\_\_\_\_

67

ge No. <sup>32</sup>P 2633 (into the anchor primer)  
follow P. 53 except use more <sup>32</sup>P ATP

~26% primer  
have ATP is  
100% efficient  
in labeling

|                              |              |              |   |   |   |                      |
|------------------------------|--------------|--------------|---|---|---|----------------------|
| 2633                         | 159 $\mu$ M  | 1 $\mu$ l    | ✓ | ✓ | ✓ | (159 $\mu$ M primer) |
| <sup>32</sup> P $\gamma$ ATP | 6000 Ci/mmol | 25 $\mu$ l   | ✓ | ✓ | ✓ | (41.8 $\mu$ M ATP)   |
| 10 mCi/ $\mu$ l              | 10-21-94     |              |   |   |   |                      |
| (11.67 $\mu$ M ATP)          |              |              |   |   |   |                      |
| 5X Kinase buffer             |              | 675          | ✓ | ✓ | ✓ |                      |
| PNK 50 $\mu$ l               |              | 0.25 $\mu$ l | ✓ |   |   |                      |
|                              |              | 33.75        |   |   |   |                      |

dry down  
1106 beads  
10  $\mu$ l H<sub>2</sub>O  
1  $\mu$ l 34P dGTP  
15' 37°C  
1  $\mu$ l EDTA

37°C 30 min  $\rightarrow$  5' 55°C  $\rightarrow$  add

spin col same as P154, 7, and 145, 3

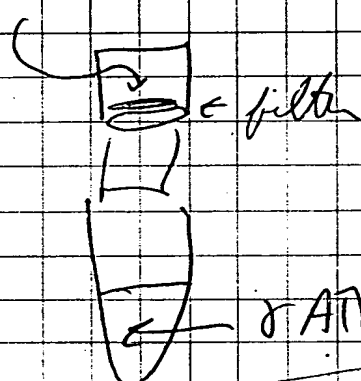
dilute <sup>32</sup>P 2633 with 100  $\mu$ l H<sub>2</sub>O (V<sub>f</sub> = 133 now)

spin in microfuge in "micron 3"

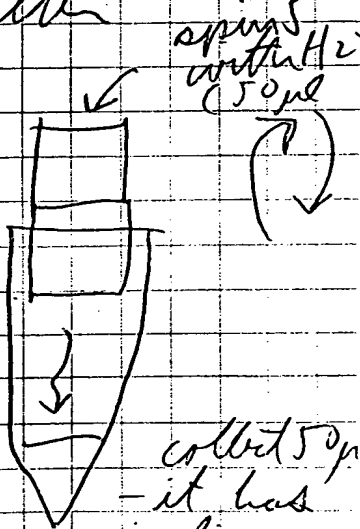
(micron # 42402) - after all venting, put

add 200  $\mu$ l more H<sub>2</sub>O and spin again

remove volume that did not enter filter



invert filter



10-24-94

Had a problem: filter kept peeling back on micron 3. Maybe 9 fold was too high on Beckman microfuge "E" model will skip separation of free ATP.

<sup>32</sup>P 2633 is diluted only 33.75 fold for  $\phi$  = 4.71  $\mu$ M

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Date

10/24/94

Inv. nted by

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Date

10-19-94  
10/24/94

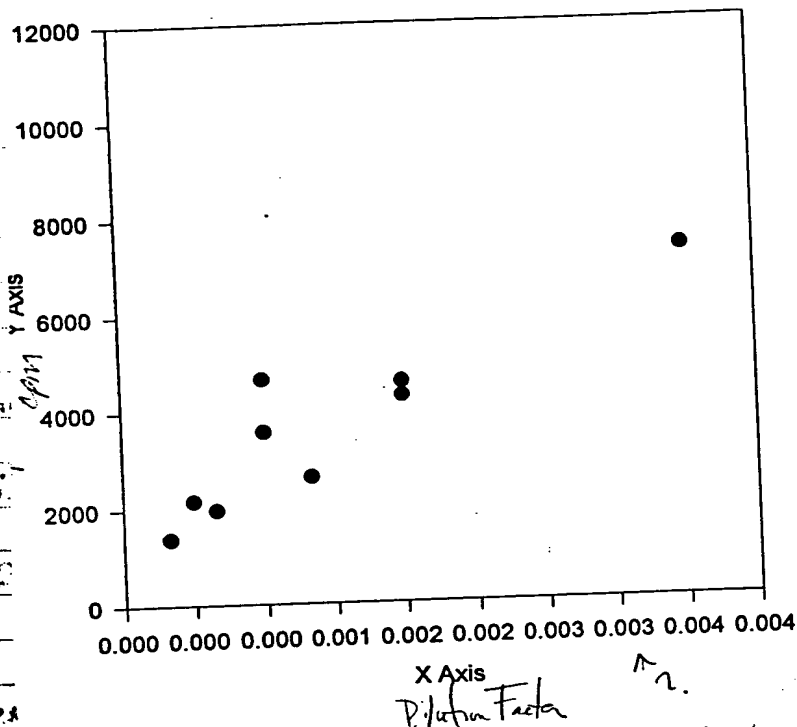
Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

04

From Page No. \_\_\_\_\_

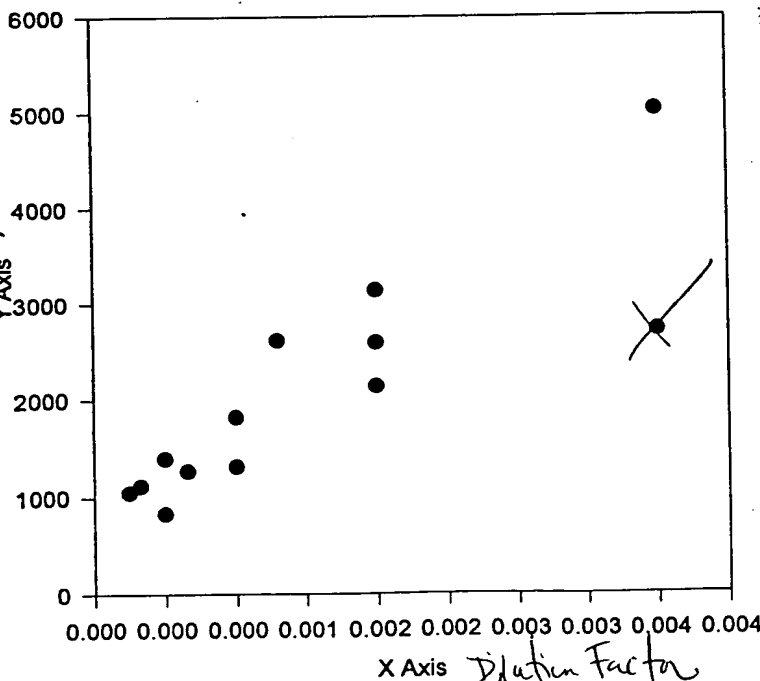


|    |          |                       |
|----|----------|-----------------------|
| 1  | 78.00    |                       |
| 2  | 1374.00  | $3.3 \times 10^{-4}$  |
| 3  | 1962.00  | $6.6 \times 10^{-4}$  |
| 4  | 2618.00  | $1.33 \times 10^{-3}$ |
| 5  | 2154.00  | $5 \times 10^{-4}$    |
| 6  | 3560.00  | $1 \times 10^{-3}$    |
| 7  | 4262.00  | $2 \times 10^{-3}$    |
| 8  | 4660.00  | $1 \times 10^{-3}$    |
| 9  | 4556.00  | $2 \times 10^{-3}$    |
| 10 | 7268.00  |                       |
| 11 | 91772.00 |                       |
| 12 | 92240.00 | $\bar{y} = 8.9450$    |
| 13 | 84328.00 |                       |

$$8.9450(25) = 55.9 \text{ cpm/pmol} \text{ -S.A.}$$

$$\text{Factor} = 1.61 \times 10^{-5}$$

$$(1.61 \times 10^{-5})(\text{cpm})(\text{DF}) = \text{U}/\mu\text{L}$$



| 3AM | CPM1     |                      |
|-----|----------|----------------------|
| 1   | 160.00   |                      |
| 2   | 1052.00  | $2.5 \times 10^{-4}$ |
| 3   | 828.00   | $5 \times 10^{-4}$   |
| 4   | 1116.00  | $3.3 \times 10^{-4}$ |
| 5   | 1262.00  | $6.6 \times 10^{-4}$ |
| 6   | 2624.00  | $1.3 \times 10^{-3}$ |
| 7   | 1392.00  | $5 \times 10^{-4}$   |
| 8   | 1310.00  | $1 \times 10^{-3}$   |
| 9   | 3140.00  | $2 \times 10^{-3}$   |
| 10  | 1820.00  | $1 \times 10^{-3}$   |
| 11  | 2134.00  | $2 \times 10^{-3}$   |
| 12  | 5024.00  | $4 \times 10^{-3}$   |
| 13  | 2592.00  | $2 \times 10^{-3}$   |
| 14  | 2716.00  | $4 \times 10^{-3}$   |
| 15  | 78604.00 |                      |

$$\text{S.A.} \sim 48.7 \text{ cpm/pmol}$$

$$\text{Factor} =$$

Witnessed & Understood by me, \_\_\_\_\_

Date \_\_\_\_\_

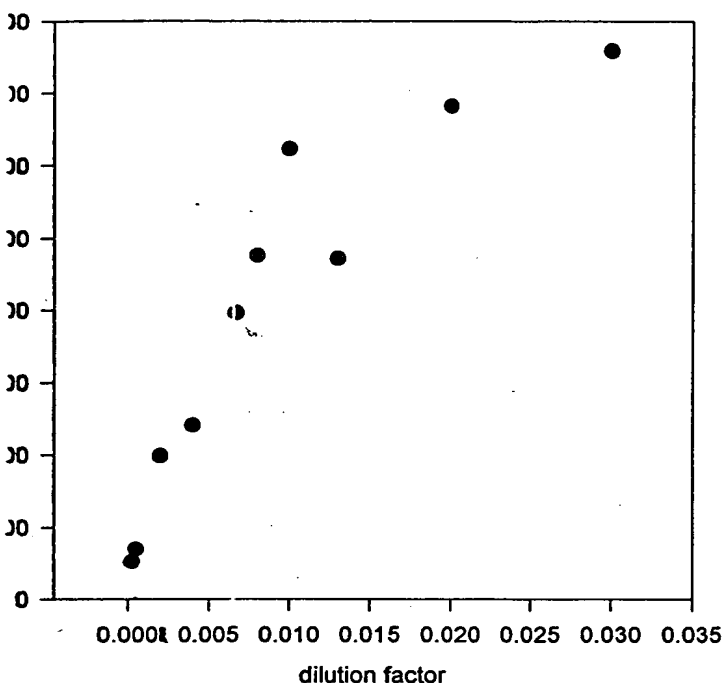
Invented by \_\_\_\_\_

Date \_\_\_\_\_

Recorded by \_\_\_\_\_

To Page \_\_\_\_\_

## Mutant Taq Titration 12/13



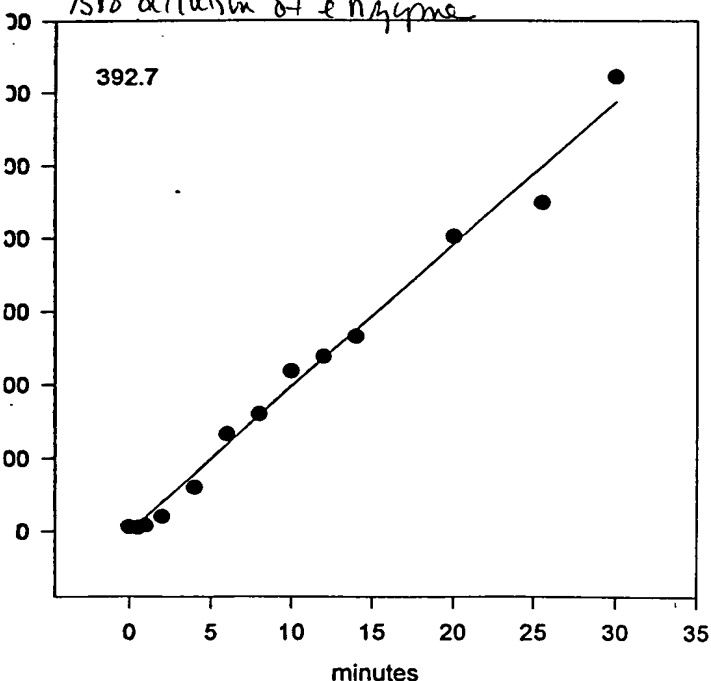
|    |          |      |      |
|----|----------|------|------|
| 1  | 12470.00 |      |      |
| 2  | 13642.00 |      |      |
| 3  | 15176.00 |      |      |
| 4  | 6342.00  |      | 32.9 |
| 5  | 7936.00  | 104  | 20.9 |
| 6  | 9428.00  | 1013 | 12.5 |
| 7  | 3970.00  | 330  | 33.0 |
| 8  | 4822.00  | 264  | 20.4 |
| 9  | 9530.00  | 211  | 21.1 |
| 10 | 3978.00  | 2    | 33.0 |
| 11 | 128.00   |      |      |
| 12 | 2624.00  | 245  | 41.1 |
| 13 | 1364.00  | 178  |      |
| 14 | 578.00   |      |      |
| 15 | 314.00   |      |      |
| 16 | 77492.00 |      |      |
| 17 | 76814.00 |      |      |
| 18 | 79502.00 |      |      |

$$\text{Factor} = 1.85 \times 10^{-5}$$

$\sim 25 \text{ V}/\mu\text{L}$

### Time course analysis of mutant Taq - 12/14

1/500 dilution of enzyme



| SAM | CPM1     |           |
|-----|----------|-----------|
| 1   | 100.00   | 8         |
| 2   | 94.00    | 30 - Time |
| 3   | 160.00   | 1         |
| 4   | 396.00   | 2         |
| 5   | 1198.00  | 4         |
| 6   | 2630.00  | 6         |
| 7   | 3170.00  | 8         |
| 8   | 4340.00  | 10        |
| 9   | 4748.00  | 12        |
| 10  | 5322.00  | 14        |
| 11  | 8060.00  | 20        |
| 12  | 9000.00  | 25        |
| 13  | 12464.00 | 30        |

Keep nice - Add 10  $\mu$ l SM  
EDTA to terminal  
types

SDO  $\mu$ l per ml  
1.1  $\mu$ l dCTP 25  
10  $\mu$ l KCO Tag-H

Heat  $274^{\circ}\text{C}$

take out 25  $\mu$ L  
aliquots at set  
intervals into  
termination  
tubes -

Spot 20 ml.

## GF/L filters

TCA wash

24 ml per mix } per row  
5  $\mu$ l enzymes } time point

↓ 740C -

25  $\mu$ l aliquots - 10  $\mu$ l EDTA.

**To Page No.**

sed & Understood by me.

Date \_\_\_\_\_

Invented by \_\_\_\_\_

Date \_\_\_\_\_

**Recorded by**

**To Page No.**

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

From Page No. \_\_\_\_\_

| SAM                | CPM1     | U/ $\mu$ l |
|--------------------|----------|------------|
| 1                  | 15576.00 | 54.5       |
| 2 $\frac{1}{500}$  | 27100.00 | 35.1       |
| 3                  | 18258.00 | 57.3       |
| 4 $\frac{1}{1000}$ | 2950.00  | 34.5       |
| 5                  | 3538.00  |            |
| 6                  | 43578.00 |            |
| 7                  | 84.00    |            |
| 8                  | 71702.00 |            |
| 9                  | 70582.00 |            |
| 10                 | 67698.00 |            |

500  $\mu$ l mix + 1.1  $\mu$ l dGTP 32-D48  $\mu$ l mix+ 1.2  $\mu$ l enzyme

10 min @ 24°C

quench w/ 10  $\mu$ l 5M EDTA + icespot 20  $\mu$ l on GF/C - TCA wash

S.A. - 43.7 cpm/pmol

$$\text{factor} = 2.0 \times 10^{-5}$$

$$(\text{cpm})(\text{factor})(\text{DF}) = \text{U}/\mu\text{l}$$

To Page No. \_\_\_\_\_

Witnessed &amp; Understood by me, \_\_\_\_\_

Date 2/2/95

Invented by \_\_\_\_\_

Recorded by \_\_\_\_\_

Date 12/94

T. neapolitana SDM

ge N .

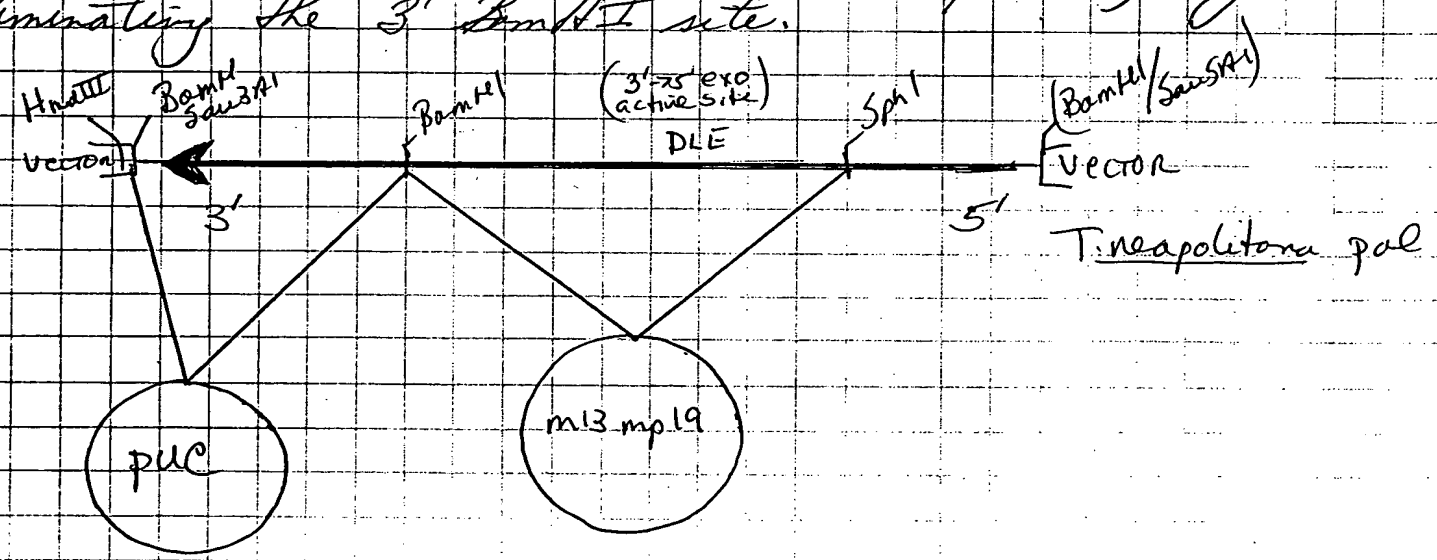
January 25, 1995 (Wednesday)

I'm BAAACK!!

after a tour of duty with Joel's group, a trip to Aulba and a few weeks vacation off I am back and ready to administer the fatal blow to this project. I will finish sequencing this gene, mutagenize it to conform to our needs, and overexpress it so people can enough enzyme to swim in it and still have money left over for a cup of coffee and a copy of the New York Times!

How's that for an opening!

First things first. Let's reclone the region of the *pal* gene we are interested in mutagenizing. Deb had I have had no success with the last clone. Secondly, let's make the subclone more user friendly by eliminating the 3' BamHI site.



strong BamHI/Sau3A  
Pcr  
clone Hd3/BamHI

make SSDNA  
D → A by SDM

To Page No. 52

|                                    |                            |                            |                 |
|------------------------------------|----------------------------|----------------------------|-----------------|
| ed & Understood by me,<br>my forgo | Date<br>1/27/95<br>4/24/95 | Invented by<br>[Signature] | Date<br>1-25-95 |
|                                    | Recorded by<br>[Signature] |                            |                 |

From Page No. 51

January 25, 1995 (Wednesday)

*T. neapolitana* / pSPORT DNA made by Michael Smith (not the Nobel laureate; the horse boy)

DIGEST SCHEME

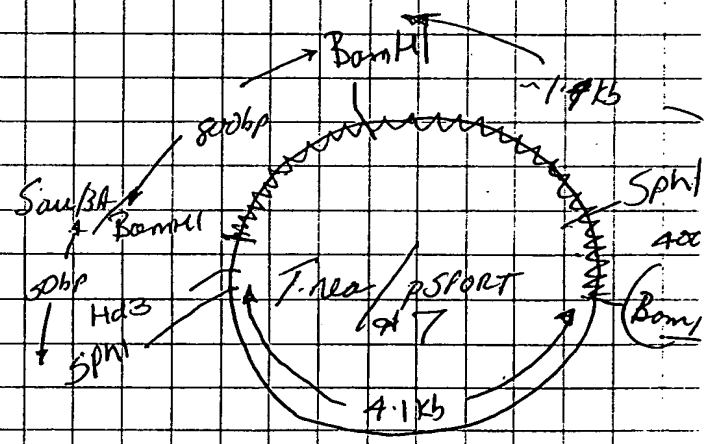
|                    | <i>T. nea</i> / pSPORT | mBap 19      | pUC 18       |
|--------------------|------------------------|--------------|--------------|
| (least 6) 10x B/R  | 15 $\mu$ l ✓           | 13 $\mu$ l ✓ | 13 $\mu$ l ✓ |
| DNA                | 2 ✓                    | 2 ✓          | 2 ✓          |
| (10 $\mu$ l) BamHI | 1 ✓                    | 3 ✓          | 3 ✓          |
| (10 $\mu$ l) SphI  | 1 ✓                    | 1 ✓          | 1 ✓          |
| Form               | 20 $\mu$ l             | 20 $\mu$ l   | 20 $\mu$ l   |
| (0.1 $\mu$ l) CAP  |                        |              | 1 $\mu$ l    |

Incubated 37°C (heat-block) 1:00 → 2:45

0.8% Agarose Gel (1XTAE)  
190 Volts

*[Redacted]*

*[Redacted]*



I forgot to run the 1 Kb ladder. What a horse boy!

Fragment *T. nea* / pSPORT? BamHI / SphI show  
be 4.5 Kb, 1.4 Kb, 0.8 Kb, 0.25 Kb.  
Perhaps something partial. Try again  
but do it separately.

Witness d & Understood by m ,

Dat

Invented by

Date

Man Tony

1/27/95

R Corded by *[Signature]*

1-25-95

To Pag N

100

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

F667X

From Page No. \_\_\_\_\_

Spin down 1.2 l cells - G-S-S - 7000 RPM - 30 min - Decant  
- Dissolve 8.6 grams cells in 25 ml Crack BFR (pg 7)

concentrate 4 x 3.5 30 sec. (4) 6 at 4.5 30 sec each

A 540 1:200 diln

Crack - .98  $\approx$  70% crack.  $\rightarrow$  Heat 15 min @ 88°C.  
Final - .13 cool 10 min / ice

ADD 0.4% PEG (1.2 ml 10% stock) - stir 15 min

Spin in 55-34 9K - 30 min - Decant sup.

7.55 gms  $\rightarrow$  ADD  $\text{NH}_4\text{SO}_4$  - 30 g / l. (40% cut) - stir 4°C 45 min

Spin down in 55-34 1/2 hr 12K - Decant sup. save for H<sub>2</sub>O  
Resuspend pellet in 8 ml BFR A - dialyze 4 hr 4°C 10 500 ml

BFR A

25 mM Tris - 7.4

8% glycerol

0.5 mM EDTA

10 mM KCl

5 mM Bmer (1.3 mM)

BFR B (high salt gradient)

Same (5) 2 M KCl

TOSO-650 Heparin

Down Sm column Equilibrate w/ A

Load at 0.5 ml/min

wash w/ 8 VTs - until baseline at 1.5 ml/min

Change dialysis buffer once -

Bump 4 ml TOSO 650 Heparin - 4M Gu HCl - 3M NaCl 10 VTs  
Wash with H<sub>2</sub>O -

Equilibrate w/ BFR A -  $\text{CTAD} = (1.4 \text{ ms})$   $\text{CTAD} = 7.1 \text{ ml} -$   
 $\text{CTAD} = 0.75 \text{ ml/min}.$

10 VT gradient A  $\rightarrow$  B - 8000 rpm

Mix - premade by H.G. - stored @ -420°C - same rxn mix as for native

With ss d & Understood by me,

Date

2/27/85

Inv nted by

*[Signature]*

Dat

12-529

R c rd d by

T Page

Page N

purpose: Amplification of PMC 9 with different amounts of Tag - titration.

Deep vent  
Tag + Deep vent

all samples discarded

Constant different concentration

using dV primers, 1 μM

200 μM dV

1 μM primer dV 2728 + 2729 (100 μM)

100 pg template diluted 100 ng / 1 → 1 ng / 1

Deep vent buffer

Titration a. Tag: 0, 0.5, 1, 1.5, 2, 2.5 and 5 U

b. Tag: Deep Vent: 1:0, 1:0.001, 1:0.005, 1:0.01, 1:0.05  
1:0.1, 1:0.2, 1:0.5, 1:1, 1:2

c. Deep Vent 0, 0.025, 0.05, 0.1, 0.5, 1, 2, 5

prepared a master mix w/o enzyme (later added separately)

Did just one of each.

H<sub>2</sub>O 116.7

10 x buffer 150

45 μl + 5 μl (enzyme + H<sub>2</sub>O)

primer 1 15

2 15

Tag: 5 U / 1 diluted to 1 U / 1 in 1 x buffer. Template 3 μl

Enzyme H<sub>2</sub>O

0 = - 5 D.V 2 U / 1 → 1 U / 1 → 0.01 U / 1 45 μl

0.5 = 0.5 4.5

0.001 U / 1

1 = 1 4 0.025 2.5

0.01

1.5 = 1.5 3.5 0.05 5

8 - 15

2 = 2 3 0.1 1

0.1

2.5 = 2.5 2.5 0.5 5

5 = 5 0 1.0 0.5

2 U / 1

2.0 = 4

5.0 = 2.5 μl

To Page No.

Seen & Understood by me,

Date

Initiated by

Date

Recorded by

11/18/94

K. Strehman

From Page No. \_\_\_\_\_

| T      | D.V   | Tag W/x   | D.V | detected in<br>1x super | Time |
|--------|-------|-----------|-----|-------------------------|------|
| Unit 1 | 0     | 1 $\mu$ l |     |                         | 16   |
|        | 0.001 |           | 1   | > .001 U/x              | 17   |
|        | 0.005 |           | 5   |                         | 18   |
|        | 0.01  |           | 1   | > .01 U/x               | 19   |
|        | 0.05  |           | 5   |                         | 20   |
|        | 0.1   |           | 1   | > .1 U/x                | 21   |
|        | 0.5   |           | 5   |                         | 22   |
|        | 1.0   |           | .5  | > 20 U/x                | 23   |
|        | 2.0   |           | 1   |                         | 24   |

- Thermocycled at 94°, 5'

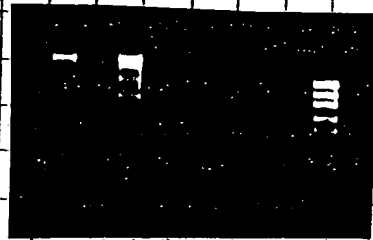
30 (94°, 30", 56°, 45 sec, 72°, 3')

- after 6 cycles noticed dNTP was not added (1) & 20 added 1  $\mu$ l of each dNTP (200  $\mu$ M / 500  $\mu$ l rx) individually each time & started again the cycling!

maybe have to repeat again.

Tag alone Deep vent alone

Tag: Deep vent



No contamination: X  
(no enzyme)

.001 .005 .01 .05 .1 .5 1 2

- so much mispriming

Repeat & in duplicate, use Lambda / Hind III marker

Witnessed & Understood by me,

Date

Invented by

Date

To Page 1

Project No. \_\_\_\_\_  
B ok No. \_\_\_\_\_

157

TNE

age No. \_\_\_\_\_

12/95

Goal: To clone the TNE 35FY (mut) into pTrc99A.

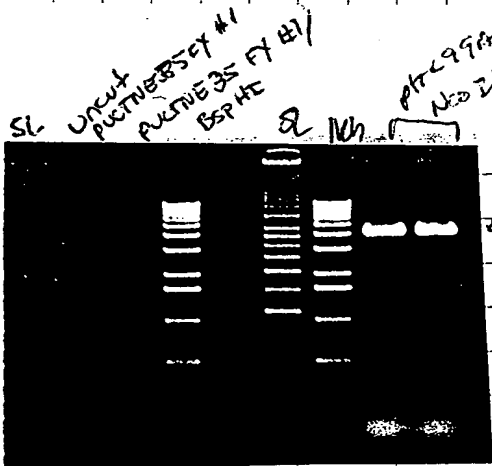
|                       |          |                  |    |
|-----------------------|----------|------------------|----|
| PUC TNE 35FY Clone #1 | 30       | pTrc99A          | 5  |
| 10xR4                 | 5        | 10xR2            | 2  |
| H <sub>2</sub> O      | 13       | H <sub>2</sub> O | 11 |
| BspHI                 | 2        | NcoI             | 1  |
|                       | 50 $\mu$ | H3               | 1  |

37°C - 1 hr.

Applied 5  $\mu$ l to -  
0.8% agarose gel  
Gel run at 180V

20  $\mu$ l

Applied to  
6.0% agarose gel  
Gel run at 180V.



cut out frag & ligate at 20°C

pTrc99A / NcoI / H3 cut looks good  
cut out 56 bp  
4174  
- 56  
4120 bp

pUC TNE 35FY #1 / BspHI gives 1 kb, 1.3 kb, + 2.7 kb frag. Therefore, BspHI cuts pUC TNE 35FY #1 3x. There must be a BspHI in the insert.

13/95

EtOH ppt. Digest.  
Dissolved in 20  $\mu$ l TE

BspHI  
5  $\mu$ l  
5  $\mu$ l 1x8T  
10  $\mu$ l  
Applied to 1 lane of - 0.8% agarose gel. Gel run at 180V

BspHI  
15  $\mu$ l DNA  
2  $\mu$ l 1xR2  
2  $\mu$ l H<sub>2</sub>O  
1  $\mu$ l H3 100:1  
20  $\mu$ l  
37°C - 1 hr.

To Page No. \_\_\_\_\_

Issued & Understood by me,

Date

Invented by

Date

L. Zhou Xu

7/14/95

Recorded by

My Long

7/13/95

<sup>32</sup>P primer for 14/ Vent  
Human spleen DNA

age N . . . <sup>32</sup>P 2633 (into the anchor primer)  
follow P. 53 except use more <sup>32</sup>P ATP

~26<sup>th</sup> primer  
now <sup>32</sup>P ATP is  
15.9 μM  
efficient  
in labeling

|                         |         |   |   |   |                  |
|-------------------------|---------|---|---|---|------------------|
| 159 μM                  | 1 μl    | ✓ | ✓ | ✓ | (15.9 μM primer) |
| 32P γ ATP 6000 Ci/mmol  | 25 μl   | ✓ | ✓ | ✓ | (41.8 μM ATP)    |
| 10 μCi/μl 10-21-94      |         |   |   |   |                  |
| (1.67 μM ATP)           |         |   |   |   |                  |
| 5x Kinase buffer        | 67.5    | ✓ | ✓ | ✓ |                  |
| PNK 50 <sup>u</sup> /μl | 0.25 μl | ✓ |   |   |                  |
|                         | 33.75   |   |   |   |                  |

Any down  
1126 ladder  
10 μl H<sub>2</sub>O  
1 μl 34P dGTP  
15' 37°C  
1 μl EDTA

37°C 30 min → 5' 55°C → add

spin col same as P154, 7, and 145, 3

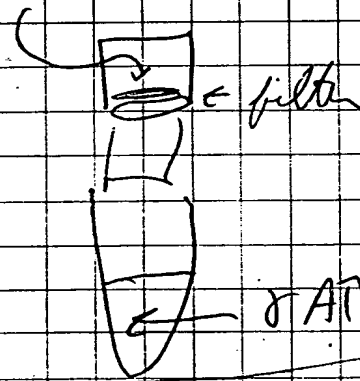
dilute <sup>32</sup>P 2633 with 100 μl H<sub>2</sub>O (Vp = 133 now)

spin in microfuge in "micron 3"

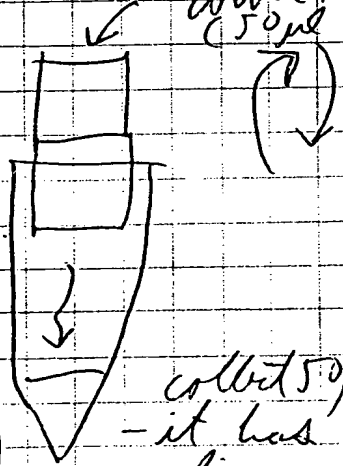
(amicon # 4240?) - after all venting, put

add 200 μl more H<sub>2</sub>O and spin again

remove volume that did not enter filter



invert filter



10-24-94

Had a problem: filter kept peeling  
back on micron 3. Maybe g force was  
too high on Beckman microfuge "E" model  
will skip separation of free ATP.

<sup>32</sup>P 2633 is diluted only 33.75 fold for  
Cp = 4.71 μM

To Page No. . . . .

Issued & Understood by me,

Researcher Robert

Date

10/24/94

Invent d by

Recorded by

Date

10-19-94  
10/24/94

108

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE 100gram Crack Tne

From Pag No. \_\_\_\_\_

Growth: 764 D1 00 IR -100g

Slurry cells in 200ml of crack buffer - lg: 2ml  
Final volume - 300ml -

Crack buffer -  
25mM Tris pH 7.4  
.1mM EDTA  
.1mM PMSF  
8% glycerol  
5mM Bme

Filter cells slurry through 4 layers  
of cheese cloth

Pass through gauze (mini) 2x @ 100

Set up 90°C heat bath before cracking cells. use -  
floor shaker -

Innucubate @ 85°C for 12 minutes - with light shaking -  
cool immediately on ice water bath ~15 min -

Spin @ 18,000 xg in GSA rotor - collect supernatant -  
- 40 min - bright yellow color -

DEI precipitation - 4:1 PEI + 50mM KCl final concentration  
add slowly over 20 minutes  
let stir an additional 45 min - spin down @ 18,000 xg  
in GSA rotor - 40 minutes -

MS (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> precipitation -

Add (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> solid to a final of 60% saturation -  
Add slowly over 30 minutes - let stir o/n @ 4°C

100mL :  $\frac{x}{250}$

To Pag N

Witnessed & Understood by me,

Date

Invented by

Dat

May Lopez

4/5/95

Rec rd d by

E. Flynn

03/29/95

*T. neapolitana* SDM

Tag No. 52

January 26, 1995 (Thursday)

DIGEST SCHEME:

|                    |         | <i>T. nea</i> /pSPORT |   | m13 mp19 (~2700bp) |   |
|--------------------|---------|-----------------------|---|--------------------|---|
| 1 (React 3)        | HOH     | 24.5 $\mu$            | ✓ | 22.5 $\mu$         | ✓ |
|                    | 10X Bfr | 3                     | ✓ | 3                  | ✓ |
|                    | DNA     | 1                     | ✓ | 3                  | ✓ |
| F-107 (104 $\mu$ ) | BamHI   | 1.5                   | ✓ | 1.5                | ✓ |
|                    | Total   | 30 $\mu$              |   | 30 $\mu$           |   |

Incubated 37°C (heat block) 8:04 → 9:08

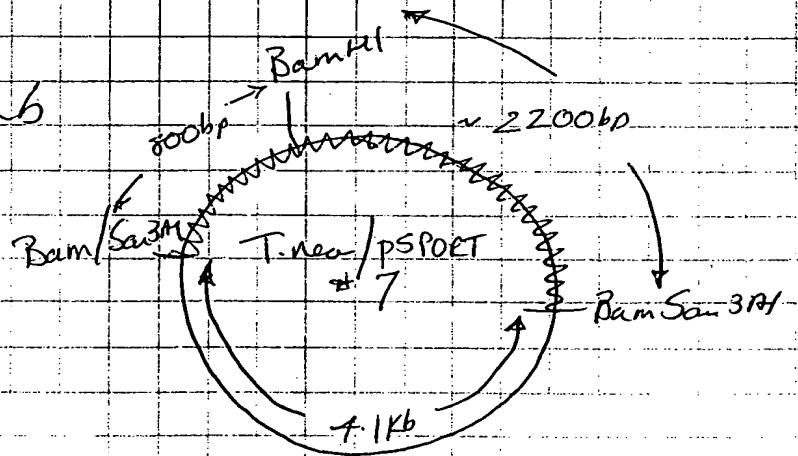
✓ 3  $\mu$  removed for analytical gel.

|          |          | <i>T. nea</i> /pSPORT | mp19 |
|----------|----------|-----------------------|------|
| DIGEST   | 27 $\mu$ | ✓                     | ✓    |
| 1MKCE    | 2        | ✓                     | ✓    |
| HOH      | 9        | ✓                     | ✓    |
| 1/4 SphI | 2        | ✓                     | ✓    |
| Total    | 40 $\mu$ |                       |      |

Incubated 37°C (heat block)  
 9:17 → 10:25

Agarose Gel (1XTAE)  
 190V x 16

Comment



To Page No. \_\_\_\_\_

Used & Understood by me,

Date

Invented by

Date

Mer Loryo

1/27/95

Recorded by

Drumf. Schmidt

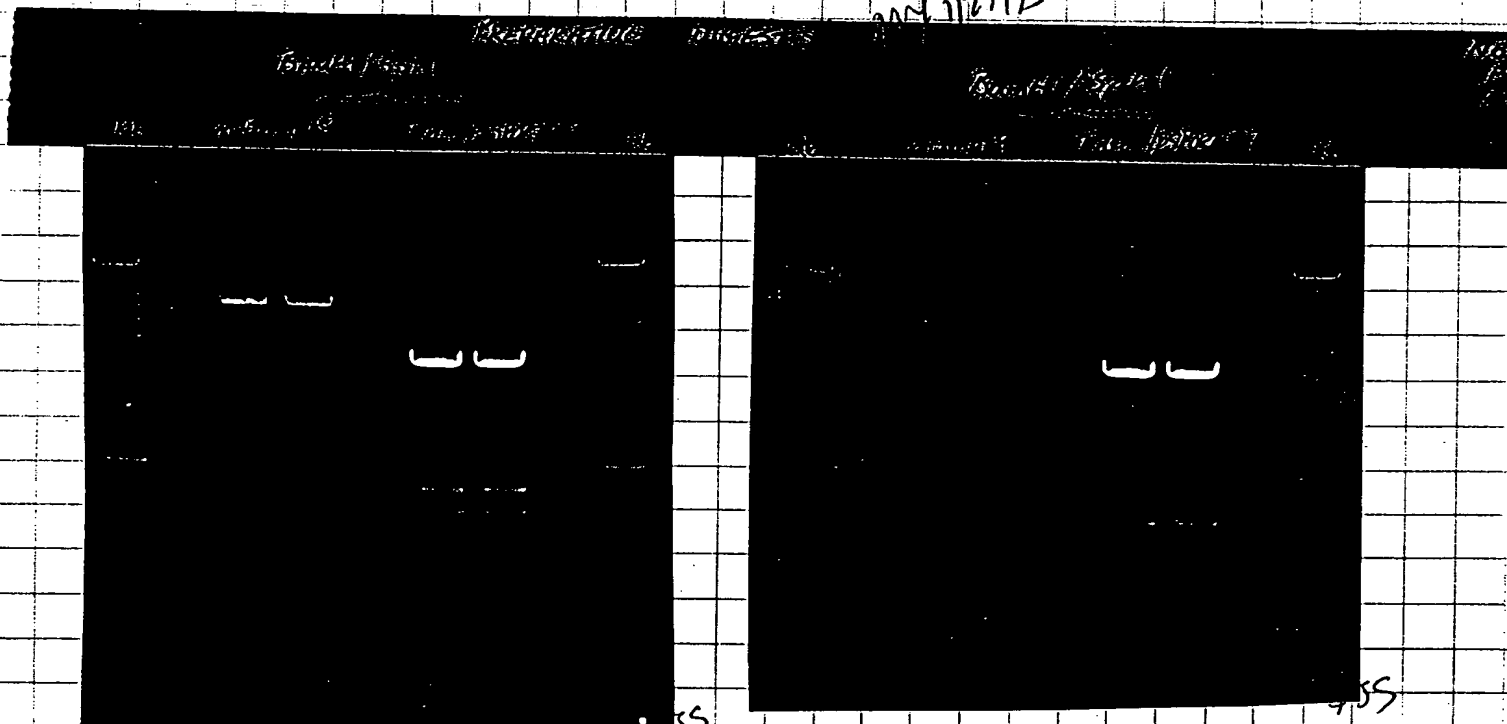
1-26-95

From Page No. 53

January 26, 1995 (Th)

0.8% Agarose Gel (1xTAE); Run at 190 Volts

JAN 27 1995



Bands extracted from the gel and placed in the same tube. The DNA was purified away from the agarose using Gene Clean as described by the manufacturer (BIO-101)

DNA eluted in 14  $\mu$ l H<sub>2</sub>O

### LIGATION SCHEME

|                     |     |            |   |
|---------------------|-----|------------|---|
| ETB 402 (Ligase)    | DNA | 14 $\mu$ l | ✓ |
| 5X Bfr              |     | 4          | ✓ |
| 14 $\mu$ l (Ligase) |     | 2          | ✓ |
| Form                |     | 20 $\mu$ l |   |

Incubated 22°C (room-temp)  
2:15 → 3:15

→ 1  $\mu$ l ligation / 2  $\mu$ l for transformation

Witnessed &amp; Understood by me,

Date

Initiated by

Date

May Long

1/27/95

Recorded by

D. J. Khum

1-26-95

*Trineapolitona* 50M

Pr j ct No. \_\_\_\_\_

B ok No. \_\_\_\_\_

55

ag N 54

January 26, 1995 (Thursday)

DH10B Electrocompetent

20  $\mu$ l DH10B Electrocompetent Cells + 1  $\mu$ l (of a 1/3 dilution; Serp 54)

2.5 KV

1ml LB, 37°C air shaker 20 min

→ 10% applied to 1B plate in 4ml Soft Agar (0.7%)  
90% + IPTG (1mM) and X-gal 100  $\mu$ l of 4%

incubated 37°C incubate

1/27/95

To Page No. \_\_\_\_\_

Used & Understood by m ,

Dat

Invented by

Date

May Longo

1/27/95

Recorded by

Dr. J. Schmidt

1-26-95

Page No. \_\_\_\_\_

type: pMC9 amplification, using 40 primers # 2722 & 2729

by titration: buffers / KlenTog  
 / Deepvent

w/ 100 dNTP

100 primer each

2 pg template pMC9 / Act II

MM Mg

cycling:  
 20 (94°, 30", 56°, 30", 72°, 3")

prepared 15 x w/o enzyme → added separately in 1x buffer

|                  |       |         |     |        |      |
|------------------|-------|---------|-----|--------|------|
| x buffer (D.V)   | 75    | (K7) 75 | 5   | } 50/λ | 1 ml |
| dNTP             | 15    | 15      | 2.5 |        | .5   |
| primer 1         | 7.5   | 7.5     | 2   | } 10/λ | 2    |
| " 2              | 7.5   | 7.5     | 1.5 |        | 1.5  |
| Mg               | —     | 15.0    | 1   | } 10/λ | 1    |
| template         | 3.0   | 3.0     | .5  |        | .5   |
| H <sub>2</sub> O | 642.0 | 627.0   | 0   |        | 0    |

distributed 50 µl / tube added enzyme.

|   |    |    |          |    |    |
|---|----|----|----------|----|----|
| 2 | 1  | 2  | (tube #) | 16 | 17 |
| — | 3  | 4  |          | 18 | 19 |
| — | 5  | 6  |          | 20 | 21 |
| 5 | 7  | 8  |          | 22 | 23 |
| — | 9  | 10 |          | 24 | 25 |
| 5 | 11 | 12 |          | 26 | 27 |
| — | 13 | 14 |          | 28 | 29 |

0.01 15 30  
 Deepvent mix

↑  
 Deepvent buffer KlenTog buffer

T Page No. \_\_\_\_\_

Is d &amp; Understood by m ,

Date

Invented by

Date

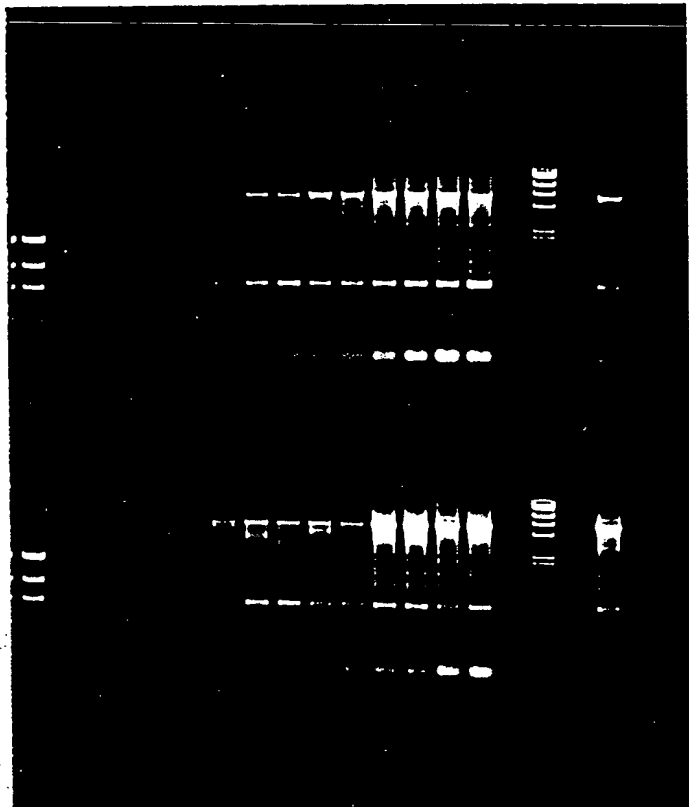
Record d by

11/22/94

K. Silberman

From Pag No. \_\_\_\_\_

0 .5 1 1.5 2 2.5 5



0 .5 1 1.5 2 2.5 5 1:0.01 max

Tag like above.

← D.V. buffer

← K.T. buffer

Result: more product with increasing amounts of Tag expected.

- K.T. B / 1U better than D.V. / 1U
- 1:0.01 better than 1U Tag alone.
- K.T. B more product than D.V. buffer
- But lot of mispriming - adjust the cycling conditions

T Pag N

Witnessed &amp; Understood by me,

Date

Invented by

Dat

Record d by

11/22/94

K. Subramaniam

TNE

Page N. \_\_\_\_\_

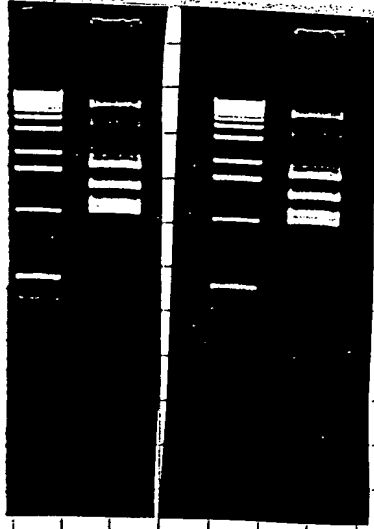
4/95

7/17/95  
 DNY

puc TNE 35EX / BspH12 → ETOH ppt. → Dissolved in 20  $\mu$ l 1x,  
 buffer. 2  $\mu$ l of H3 (100:1) was added. 37°C - 1h.  
 applied to 1 lane of a 1% LMP agarose gel.  
 Gel run at 180V.

1x  
 7/20/95

cut the 200bp frag out &  
 luge at -20°C.



95

used the ~~phenol~~ phenol extraction method to purify DNA.  
 Dissolved in 10  $\mu$ l TE.

To Page No. \_\_\_\_\_

Read & Understood by me,

Lizhu Yu

Date

7/20/95

Invented by

Recorded by

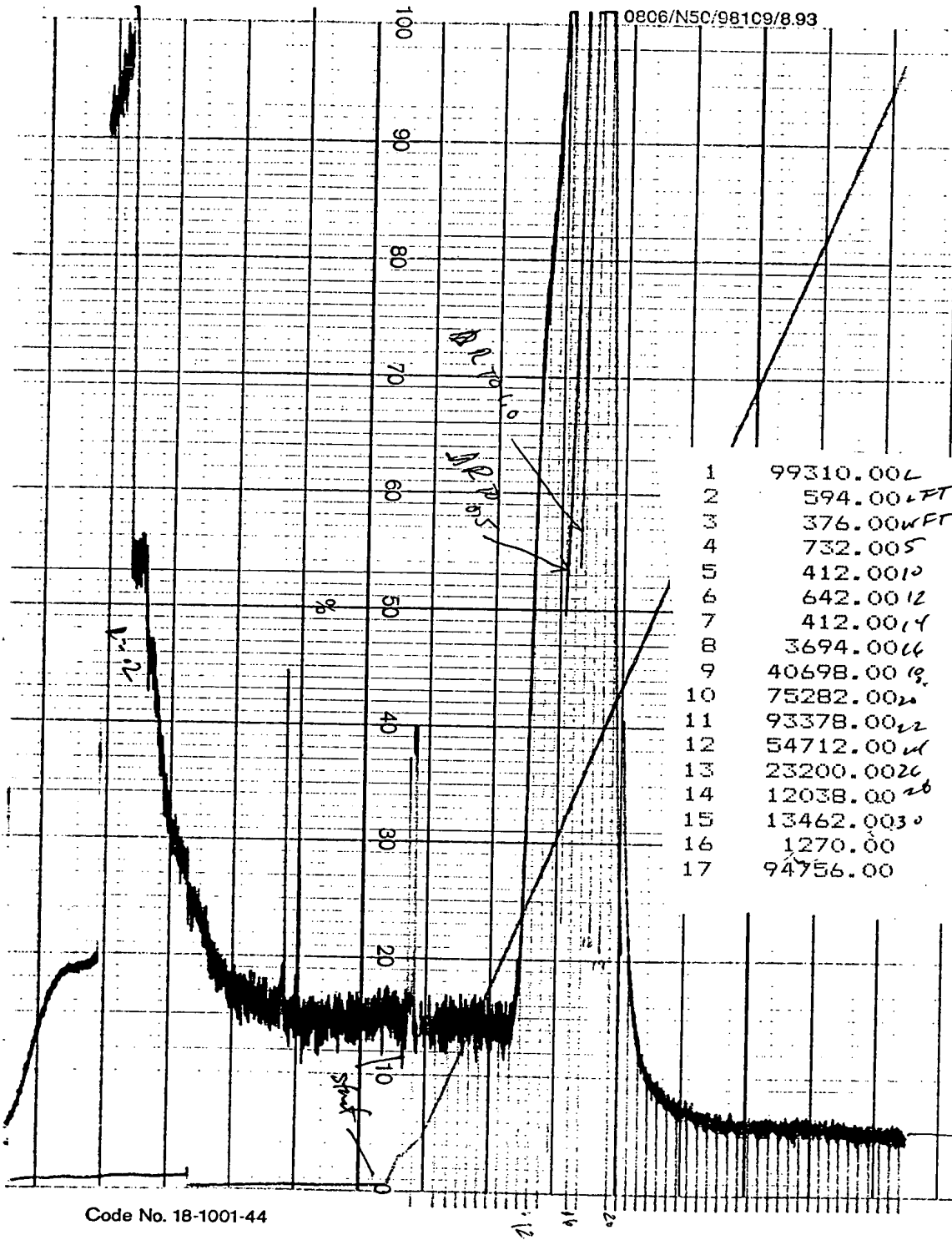
Ming Long

Date

7/18/95

TOSO - Hg 650(m)

3g N .



0806/N50/98109/8.93

|    |            |
|----|------------|
| 1  | 99310.00L  |
| 2  | 594.00LFT  |
| 3  | 376.00WFT  |
| 4  | 732.005    |
| 5  | 412.0010   |
| 6  | 642.0012   |
| 7  | 412.0014   |
| 8  | 3694.0016  |
| 9  | 40698.0018 |
| 10 | 75282.0020 |
| 11 | 93378.0022 |
| 12 | 54712.0024 |
| 13 | 23200.0026 |
| 14 | 12038.0028 |
| 15 | 13462.0030 |
| 16 | 1270.00    |
| 17 | 94756.00   |

74°C - 8 min  
 24 WFT  
 1st sample - STOP  
 2nd sample - STOP  
 3rd sample - STOP  
 4th sample - STOP  
 5th sample - STOP  
 6th sample - STOP  
 7th sample - STOP  
 8th sample - STOP  
 9th sample - STOP  
 10th sample - STOP  
 11th sample - STOP  
 12th sample - STOP  
 13th sample - STOP  
 14th sample - STOP  
 15th sample - STOP  
 16th sample - STOP  
 17th sample - STOP  
 18th sample - STOP  
 19th sample - STOP  
 20th sample - STOP  
 21st sample - STOP  
 22nd sample - STOP  
 23rd sample - STOP  
 24th sample - STOP

- 1 5
- 2 5
- 3 5
- 4 5
- 5 10
- 6 12
- 7 14
- 8 16
- 9 18
- 10 20
- 11 22
- 12 24
- 13 26
- 14 28
- 15 30
- 16 32
- 17 34

Pool - 1779  
 - Daily 1000  
 B.P. 10.5

Code No. 18-1001-44

To Page No. \_\_\_\_\_

Read & Understood by me,

Date

Invented by

Date

Recorded by

10

2/27/96

2-10-96

# Human spleen DNA

Project N \_\_\_\_\_

Exhibit 4

Appl. No. 09/558,421

B k No. \_\_\_\_\_

67

age N \_\_\_\_\_  $^{32}P$  2633 (into the anchor primer)  
 follow P. 53 except use more  $^{32}P$  ATP

|                    |              |              |   |   |   |                      |   |   |
|--------------------|--------------|--------------|---|---|---|----------------------|---|---|
| iso 2633           | 159 $\mu M$  | 1 $\mu l$    | ✓ | ✓ | ✓ | (159 $\mu M$ primer) | ② | ~26% primers<br>have ATP in<br>100% efficiency<br>in labeling |
| $^{32}P$ ATP       | 6000 Ci/mmol | 25 $\mu l$   | ✓ | ✓ | ✓ | (41.8 $\mu M$ ATP)   |   | Any down<br>1106 ladder                                       |
| 10 mCi/ $\mu l$    | 10-21-94     |              |   |   |   |                      |   | 10 $\mu l$ H <sub>2</sub> O                                   |
| (1.67 $\mu M$ ATP) |              |              |   |   |   |                      |   | 1 $\mu l$ $^{32}P$ OP   |
| 5x Kinase buffer   |              | 675          | ✓ | ✓ | ✓ |                      |   | 15' 37°C  |
| PNK 50 $\mu l$     |              | 0.25 $\mu l$ | ✓ |   |   |                      |   | 1 $\mu l$ EDTA  |
|                    |              | 33.75        |   |   |   |                      |   |   |

37°C 30 min → 5' 55°C → add

spin col same as P. 54, 7, and 145, 3

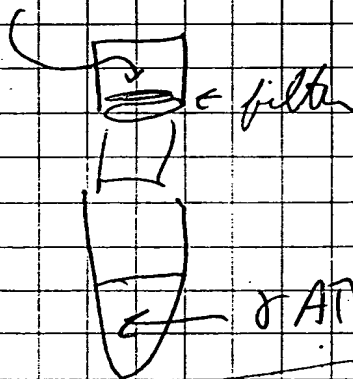
dilute  $^{32}P$  2633 with 100  $\mu l$  H<sub>2</sub>O ( $V_p = 133$  now)

spin in microfuge in "micron 3"

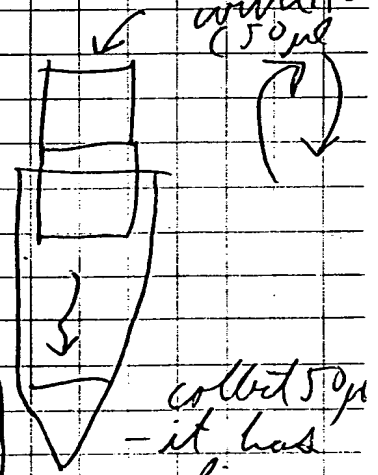
(amicon # 4240?) - after all went in, put

add 200  $\mu l$  more H<sub>2</sub>O and spin again

remove volume that did not enter filter



invert filter



10-24-94

Had a problem: filter kept peeling back on micron 3. Maybe g force was too high on Beckman microfuge "E" model will skip separation of free ATP.

$^{32}P$  2633 is diluted only 33.75 fold for  $C = 4.71 \mu M$

To Page No. \_\_\_\_\_

Read & Understood by me,

Steven A. Pokany

Date

10/24/94

Invented by

Recorded by

Date

10-19-94  
10/24/94

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE 13.5 Kb long PCR

From Page No. \_\_\_\_\_

 $^{32}P$  2633 4.71  $\mu M$ ①  
✓ 4.7  $\mu l$ ②  
4.7  $\mu l$ 0.2  $\mu l$ 2628 old 199  
dilute to 10  $\mu M$ ✓ 2.2  $\mu l$  →0.2  $\mu l$ 80 ng/ $\mu l$  Human  
spleen DNA✓ 1.1  $\mu l$  →(80 ng/ $\mu l$ )

4 dNTPs 10 mM each

✓ 2.2  $\mu l$  →200  $\mu M$ 

pol mix

Vent 2  $\mu l$ 0.52  $\mu l$ Tf1 1  $\mu l$ 15  $\mu l$ Vf = 15.5  $\mu l$  →✓ 1.36  $\mu l$ 

4.08

Total  
(1.32 unit  
0.087  $\mu l$  in ①  
more in ② =  
0.28  $\mu l$  Vent5X buffer  
Cheng

✓ 22 →

Mg (OAc)<sub>2</sub>  
12 mM

✓ 11 →

Cp = 1.2

H<sub>2</sub>O

32.2/1.2 =

65.44

75.34

Vp = 110  $\mu l$ 

62.7

72.6

110  $\mu l$ remove 10  $\mu l$  to 2  $\mu l$  0.2 M EDTA at 0 cycles.  
remove 10  $\mu l$  at 5, 10, 15, 20, 25, 30, 36

program

139

15", 94°C → 20 min, 68°C

140

10", 72°C

141

1", 94°C

142 = 141, 139, 140, 4

started at 8:16 AM  
20.5 min/cycleneed 12 hr, 20 min to  
complete so expect to end  
at 8:40 - 9:00 PM

T Page No.

Witnessed &amp; Understood by me,

Deena Golov

Date

10/24/94

Invent d by

Record d by

Date

10-24-94

Proj ct No. \_\_\_\_\_

B ok No. \_\_\_\_\_

69

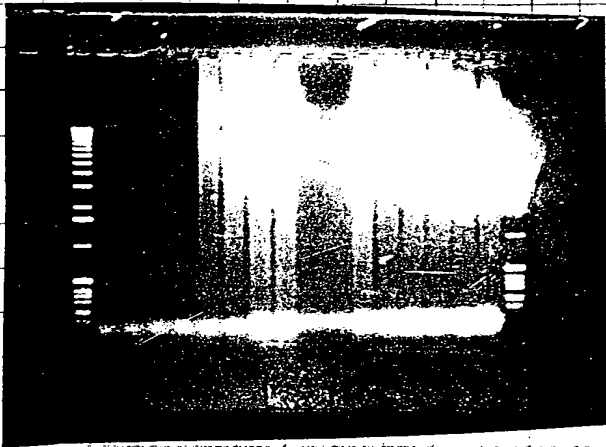
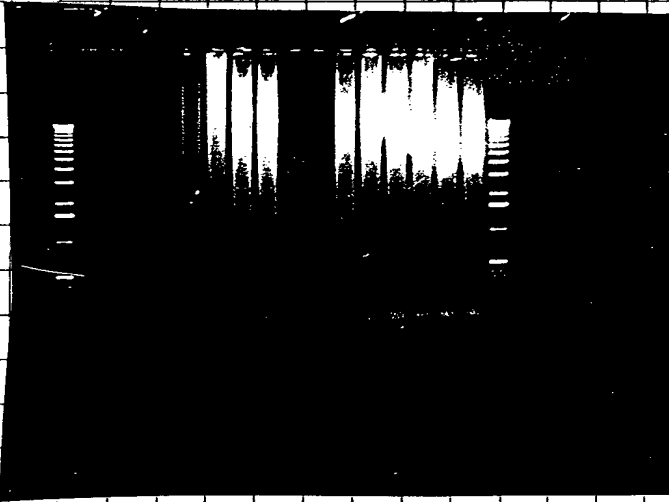
ig N — 8% again same as P.56

Tf) :

1.33

4

05 10 15 20 25 30 36 05 10 15 20 25 30 36



To Pag No. \_\_\_\_\_

ed & Understood by me,

Date

10/24/94

Invent d by

Recorded by

Dat

Eric Bokup

Project No. \_\_\_\_\_  
Block No. \_\_\_\_\_

The over Heparin 40mL column.

Tag No. \_\_\_\_\_

Spin  $(\text{NH}_4)_2\text{SO}_4$  sol'n - @ 18,000 x g 40 minutes -  
in GSA rotor -

Save supernatant -  
Save pellets -

Store one pellet in -20°C - process the other pellet 2

2: pellet 1 slightly greater than half -  $\sim 3/5$

pellet 2 slightly less than half -  $\sim 2/5$

Resuspend pellet in 20 mL of Buffer 1 -

Buffer 1

5 mM Tris pH 7.5  
3.1 glycerol  
40 mM KCl  
5 mM Bme  
1 mM PMSF

dialyze - against Buffer 1 for  $\sim 8$  hrs -  
Exchange buffer 4 times -

heparin column - use prepacked Heparin from A.G. -  
 $\sim 40$  mL column - bump w/ Buffer + KCl -  
wash w/  $\text{H}_2\text{O}$  -

Previously A.G. used 3 mL Heparin a  
5 gram crack

Direct scale up =  $\frac{3}{5} = \frac{4}{5} = 30$  mL Heparin  
 $\sim 50$  g

equilibrate w/ Buffer 1  $\rightarrow$  (Note: made 20 mM KCl -)

To Page No. \_\_\_\_\_

|   |                |                      |                  |
|---|----------------|----------------------|------------------|
| Read and Understood by me,<br>May forso | Date<br>4/5/95 | Invented by E. Hyman | Date<br>03/30/95 |
|   |                | Recorded by          |                  |

From Page No. \_\_\_\_\_

Conductivity of Load - 2.8 mS - after ~8 hrs of dialysis

Notice a small precipitate matter in dialysis tube -  
Spin down in SS-34 - 18,000 x g - 10 minutes -  
same pellet - small + white -

① Load - 21 mL of sample - 75 ~~mm~~ mL/min - collect FT -

② Wash - 2 V<sub>t</sub> of Buffer 1 - collect <sup>7.5</sup> 8 mL fractions  
1 mL/min

③ Gradient - Buffer 1 to Buffer 2 - 25 mM Tris pH 7.5  
8% glycerol  
5 mM BME  
1 mM PMSF  
2 M KCl

10 V<sub>t</sub> - 400 mL gradient - linear - 1 mL/min -  
collect 7.5 mL fractions -

④ Wash w/ 2 V<sub>t</sub> Buffer 2 1 mL/min - 7.5 mL fractions -

Let column run O/N -

Note: Next time gradient should be much shallower - 1 M KCl -

To Page N

Witness d &amp; Understood by me,

Date

Invented by

Dat

-Mary Long

4/5/95

Recorded by

03/31/95

56

Project No. 20222

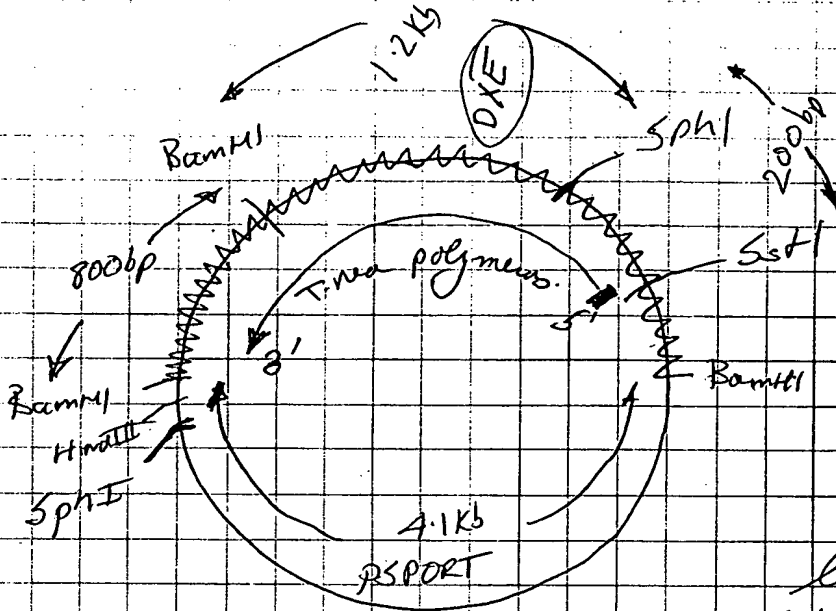
Book No. 3884

TITLE

T. neapolitana 30M

From Page No. 33

February 7, 1995 (Tuesday)



I can clone & SphI fragments into m13mp19 and let m13 determine which direction is best suited.

I can subclone the fragment into an expression vector with SphI / HindIII.

### DIGEST SCHEME

|                 | T. nea RSPORT | (272 ng/μl) m13mp19 |           |
|-----------------|---------------|---------------------|-----------|
| (Ready) 10x B/R | 21 μl         | 20 μl               | 9:42 am → |
| DNA             | 3             | 3                   | 10:00     |
| (100 μl) SphI   | 3             | 3                   |           |
| (0.7 μl) CAP    | 0             | 1                   |           |
| TOTAL           | 30 μl         | 30 μl               | 9:46 am → |

Run on 0.8% Agarose Gel at 75 V/1h

To Page

Witnessed & Understood by me,

May Longo

Date

2/16/95

Inv nt d by

Recorded by

[Signature]

Date

2-7-95

*T. neapolitana* SOM

Project N 20222

B ok No. 3884

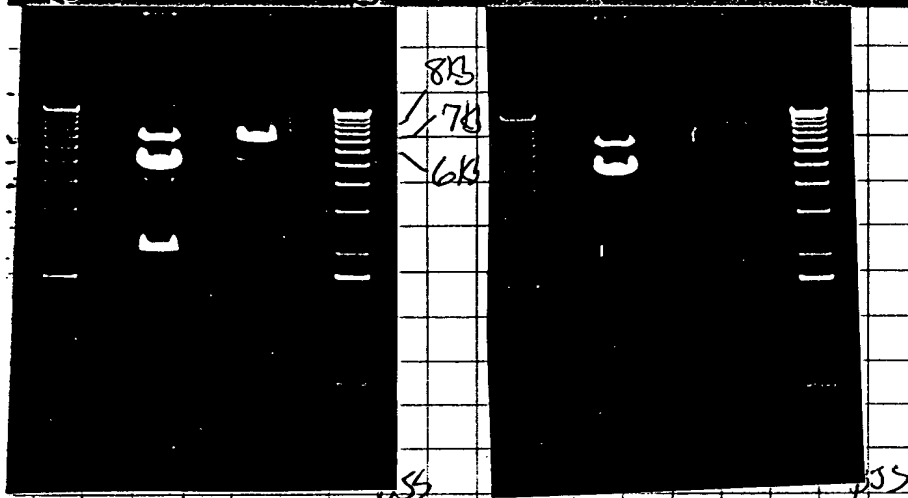
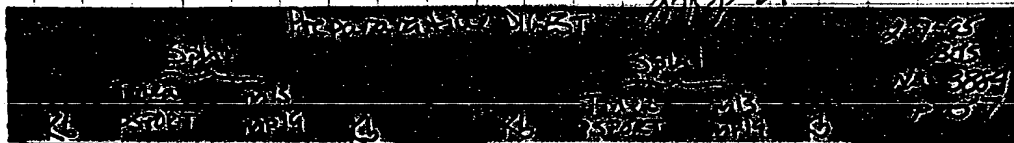
57

N 56

February 7, 1995 (Tuesday)

0.8% Agarose Gel (1X TAE); 75 V 16

2/16/95



Bands extracted from the gel and the DNA purified away from the agarose using Gene Clean as described by BIO-101.

DNA eluted in 14  $\mu$ l HOH

LIGATION SCHEME

|                |    |         |   |
|----------------|----|---------|---|
| HOH            | -  | $\mu$ l |   |
| 5X Bfr         | 4  |         | ✓ |
| DNA            | 14 |         | ✓ |
| WAT DNA Ligase | 2  |         | ✓ |
| Total          | 20 | $\mu$ l |   |

Incubated 3:23 pm  $\rightarrow$  4:03 at room-temperature ( $\sim 22^{\circ}\text{C}$ )

3  $\mu$ l removed for transformation

152 F' IQ Competent All Transformation  
 0.1  $\mu$ l competent cells + 3  $\mu$ l ligation (see above)  
 2 min on ice, 35 seconds at  $42^{\circ}\text{C}$  water bath  
 1. and 90  $\mu$ l applied to LB + No Antibiotic plates in 4 ml 0.7% Top Agar + 100  $\mu$ l 2% X-Gal + 10  $\mu$ l 100 mM IPTG  
 incubated 16 hours at  $37^{\circ}\text{C}$  incubator

To Page No. 58

|   |                 |  |               |
|---|-----------------|--|---------------|
| d & Understood by me,<br><i>Neeraj Soni</i> | Date<br>2/16/95 | Invented by<br><i>Tracy J. Schmidt</i> | Dat<br>2-7-95 |
|---|-----------------|--|---------------|

11/21/94

PMCP / Tag + D.V.

Page No. \_\_\_\_\_

Tag 1 U + Deep Vent different amount in PCRing  
PMCP.

Deep Vent buffer 22x KlenTag buffer.

|                  |       |       |                 |
|------------------|-------|-------|-----------------|
| 10x buffer       | 110   | 110   | 200 µl dNTP     |
| dNTP             | 22    | 22    | 1 µl primer     |
| Mg               | —     | 22    | 200 µg Template |
| primer 1         | 11    | 11    | 1 µl 20x        |
| 2                | 11    | 11    |                 |
| Template         | 4.4   | 4.4   |                 |
| H <sub>2</sub> O | 895.6 | 873.6 |                 |

added 1 U Tag in 1 µl in 1x buffer.

added different amount of Deep Vent in 2 µl in 1x buffer  
either D.V. or RT buff.

\* Tubes.

| Tag | Deep Vent | Deep Vent buffer | K. T. buffer |
|-----|-----------|------------------|--------------|
| 0   | 0         | 1 2              | 22 23        |
| 1   | 0         | 3 4              | 24 25        |
|     | .001      | 5 6              | 26 27        |
|     | .005      | 7 8              | 28 29        |
|     | .01       | 9 10             | 30 31        |
|     | .05       | 11 12            | 32 33        |
|     | .1        | 13 14            | 34 35        |
|     | .5        | 15 16            | 36 37        |
|     | 1         | 17 18            | 38 39        |
|     | 2         | 19 20            | 40 41        |

94°, 3' 21' 42'  
94°, 30", 56°, 30", 72°, 3' 1 : 0.01 mix

started the cycling file instead of stop file - 2 cycles down  
before changing to stop file. To Page No. \_\_\_\_\_

Read &amp; Understood by me,

Date

Invented by

Date

Recorded by

11/22/94

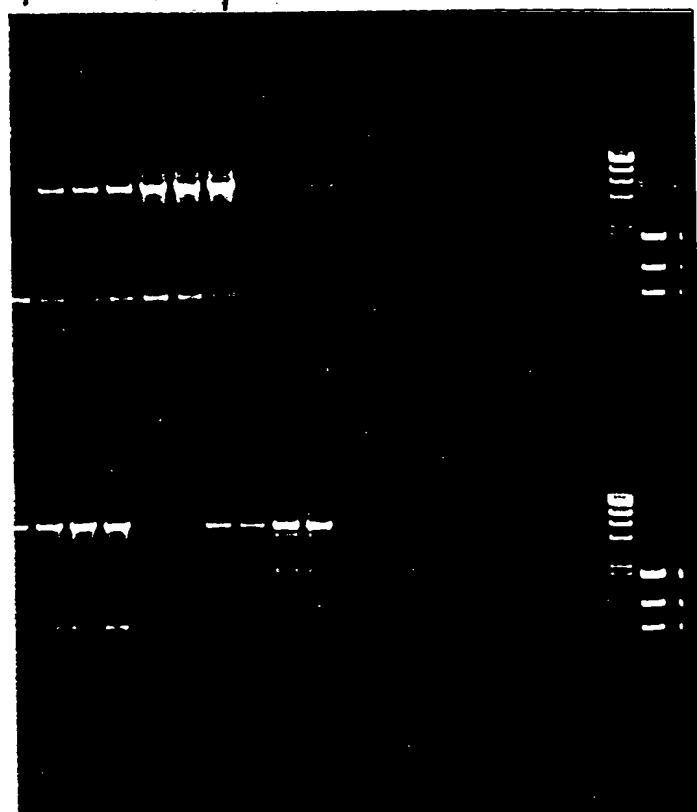
J. S. S. S. S.

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

From Page No. \_\_\_\_\_



Tag: devent  
libation

← D.V. Buffer - up to  
1: 0.01 ok

← R.T. Buffer can go  
up to 1: 0.05 but  
this can't D.V. more  
mispriming

1: 0.001 | 1: .01 | 1: .05 | 1: .1 | 1:2

1: .005 | 1: .05

- ? maybe didn't add any Tag - make primer to next
- mispriming still there - check annealing temp.
- Try again with new D.Vent.
- Increasing D.V. over 0.05 = having D.V. alone.  
Tag effort nil.

discarded

12/19/94

To Page N

Witnessed &amp; Understood by me,

Dat

Invented by

Dat

Recorded by

K. Sturman

11/22/94

TNE

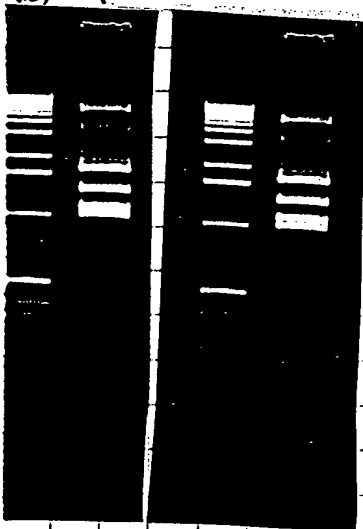
Page N \_\_\_\_\_

1/95

7/14/95  
GMY

puct TNE 35FX / BspH E → ETOH ppt → Dissolved in 20ul 1x1  
bottle. 2ul of H3 (100%) was added 37°C - 1hr  
applied to 1 lane of a 1% LMP agarose gel  
Gel run at 180V.

1/1  
7/20/95



cut the 200bp frag out &  
ligate at -20°C.

← 200bp frag

25

Used the <sup>GMY</sup> phenol extraction method to purify DNA.  
Dissolved in 10ul TE.

To Page No. \_\_\_\_\_

sed & Understood by me,

Date

Invented by

Date

Litu Xu

7/20/95

Recorded by

My Long

7/18/95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE SDS gel Thermostable pols

72

From Page No. \_\_\_\_\_

| mw  | 1   | 2   | 3   | 4   | 5 | 6   | 7   | 8            |
|---|-----|-----|-----|-----|---|-----|-----|--------------|
| TfI epicenter<br>lot TFS0809A<br>5 $\mu$ /ml                        | 100 |     |     |     |   |     |     | ✓            |
| TfI MBR 5 $\mu$ /ml<br>cat # 111202 (TfI)<br>lot 40104              |     | 100 |     |     |   |     |     | ✓            |
| Tth MBR 5 $\mu$ /ml<br>lot 21021, cat 1115-02                       |     |     | 100 |     |   |     |     |              |
| FTth Perkin Elmer, 2.5 $\mu$ /ml<br>cat N808-0007<br>lot 9189       |     |     |     | 170 |   |     |     |              |
| sequen therm epicenter 5 $\mu$ /ml<br>lot 0140303                   |     |     |     | 100 |   |     |     |              |
| Vent (NEB) 2 $\mu$ /ml<br>lot 17, assayed 7/94                      |     |     |     |     |   | 250 |     |              |
| Deep Vent (NEB) 2 $\mu$ /ml<br>lot 4, assayed 8/94                  |     |     |     |     |   |     | 250 |              |
| RTag EKBTI 40 $\mu$ /ml<br>H <sub>2</sub> O                         |     |     |     |     |   |     |     | 1.25 $\mu$ l |
| TCA 15%   |     |     |     |     |   |     |     |              |
| (see P 50-7 for TCA ppt)  |     |     |     |     |   |     |     |              |
| 30' ice   |     |     |     |     |   |     |     |              |
| 10' microfug at 4°C, remove supernatant                             |     |     |     |     |   |     |     |              |
| Vortex pellet in ice cold acetone, microfug 10', remove supernatant |     |     |     |     |   |     |     |              |
| dry 37°C 25', resuspend in 60 $\mu$ l 1x cracking buffer            |     |     |     |     |   |     |     |              |

Witnessed & Understood by me,  
Deena Polansky

Date  
11/29/94

Invent d by  
[Signature]

Record d by

Date  
10-25-94

T Page No.

[illegible]

**To Page No.\_\_\_\_\_**

ed & Understood by me,

**Dat**

**Inv nted by**

**Date**

Ernst Polenz

11/29/94

Recorded by \_\_\_\_\_

10-25-94

Pag N \_\_\_\_\_

urpose: To amplify pMC9 with Deep Vent alone  
 tried in Deep Vent buffer only.

|                  |     |
|------------------|-----|
| 10x buffer       | 100 |
| dNTP             | 20  |
| Mg               | —   |
| primer 1         | 10  |
| 2                | 10  |
| Template         | 4   |
| H <sub>2</sub> O | 846 |

|        |          |
|--------|----------|
| 200 µl | dNTP     |
| 2 mM   | Mg       |
| 1 µl   | primer   |
| 200 µl | Template |

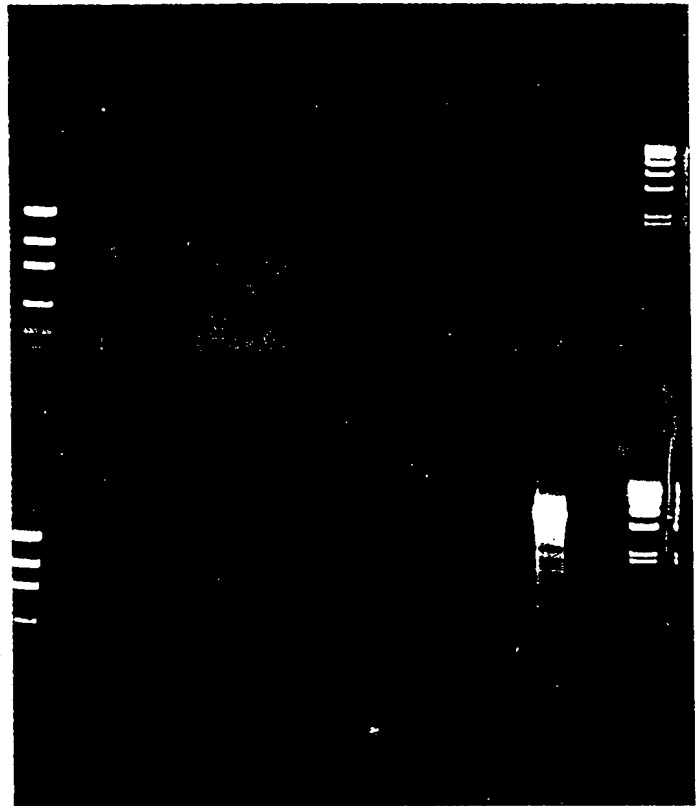
94° 30'

30( 94°, 30', 56°, 30', 72°, 30',

|      |    |    |
|------|----|----|
| 0    | 1  | 2  |
| .001 | 3  | 4  |
| .005 | 5  | 6  |
| .01  | 7  | 8  |
| .05  | 9  | 10 |
| .1   | 11 | 12 |
| .5   | 13 | 14 |
| 1    | 15 | 16 |
| 2    | 17 | 18 |

result: Once again  
 rep. Vent alone by itself  
 didn't amplify anything  
 with this dv primers.

See the other set to  
 confirm.



To Page No. \_\_\_\_\_

Issued &amp; Understood by me,

Date

Invented by

Date

Recorded by

11/22/94

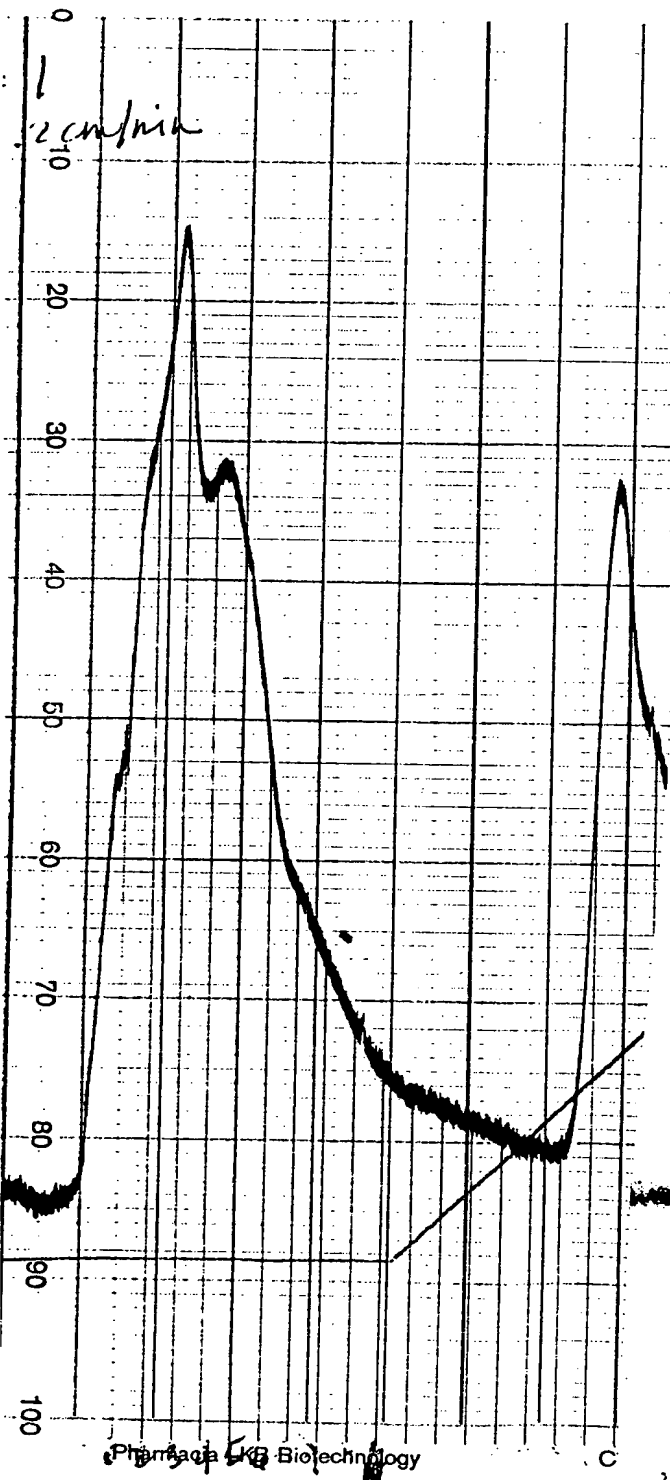
A. Sitarman

Project No. \_\_\_\_\_  
Bo k N . \_\_\_\_\_

111

40 ml Hepara

ag No. \_\_\_\_\_



3/31/95

nm  
4/5/95

To Page No. \_\_\_\_\_

|   |                    |                           |                  |
|---|--------------------|---------------------------|------------------|
| Read & Understood by me,<br><br>May Longo | Date<br><br>4/5/95 | Inv. nted by<br>E. Algran | Date<br>05/31/95 |
|   |                    | Recorded by               |                  |

From Page N \_\_\_\_

SAM CPM1

|    |           |      |
|----|-----------|------|
| 1  | 135612.00 | lead |
| 2  | 310.00    | 1    |
| 3  | 460.00    | 2    |
| 4  | 512.00    | 3    |
| 5  | 386.00    | 4    |
| 6  | 308.00    | 5    |
| 7  | 1118.00   | 6    |
| 8  | 960.00    | 10   |
| 9  | 546.00    | 15   |
| 10 | 420.00    | 16   |
| 11 | 1368.00   | 17   |
| 12 | 6588.00   | 18   |
| 13 | 45516.00  | 19   |
| 14 | 70278.00  | 20   |
| 15 | 98796.00  | 21   |
| 16 | 91534.00  | 22   |
| 17 | 109058.00 | 23   |
| 18 | 129224.00 | 24   |
| 19 | 73534.00  | 25   |
| 20 | 32032.00  | 26   |
| 21 | 13662.00  | 27   |
| 22 | 3166.00   | 28   |
| 23 | 2848.00   | 29   |
| 24 | 1910.00   | 30   |
| 25 | 1508.00   | 31   |
| 26 | 1426.00   | 32   |
| 27 | 3168.00   | 33   |
| 28 | 1278.00   | 34   |
| 29 | 840.00    | 35   |
| 30 | 516.00    | 36   |
| 31 | 119806.00 |      |
| 32 | 121684.00 |      |
| 33 | 123400.00 |      |
| 34 | 26.00     |      |
| 35 | 44.00     |      |
| 36 | 50.00     |      |

POOL

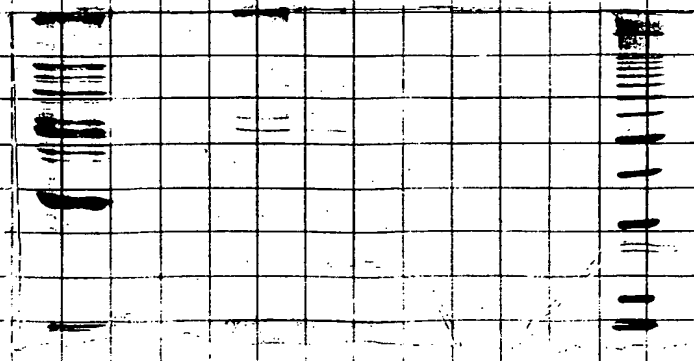
Add 22  $\mu$ l of LdCTP to 1ml of premix -  
(From A.G.)

aliquot 24  $\mu$ l to pre-labeled ependants  
add .5  $\mu$ l of sample - incubate 10min @  
72°C quench on ice + add 10  $\mu$ l of 5MED  
Spot 20  $\mu$ l on GF/C filters -  
wash w/ 10% TCA + 1% PPi - x1  
1% TCA x4  
EtOH x2

dry + count

Pool dialyzed O/N (sat). 104/01/95

Gel of Northern Fracting



4/15/95  
4/15/95

Witness d &amp; Understood by me,

Date

Invented by

Date

To Page N

Mary Long

4/15/95

R c rded by

E. Ryan

03/31/95

7 column Bradfads → P.Y. PAGE

Project No. \_\_\_\_\_

B ok No. \_\_\_\_\_

113

ag N \_\_\_\_\_

Conc (mg/ml)

|           |           |
|-----------|-----------|
| 11.274956 | Cude      |
| 0.307192  | Heat Kill |
| 1.065601  | PEI       |
| 0.819939  | AS sup    |
| 3.081949  | A Load    |
| 0.385722  | 17        |
| 0.375194  | 18        |
| 0.285862  | 19        |
| 0.329252  | 20        |
| 0.368813  | 21        |
| 0.432621  | 22        |
| 0.980098  | 23        |
| 0.798244  | 24        |
| 0.419222  | 25        |
| 0.177069  | 26        |
| 0.069870  | 27        |

- too Not a. lide enough

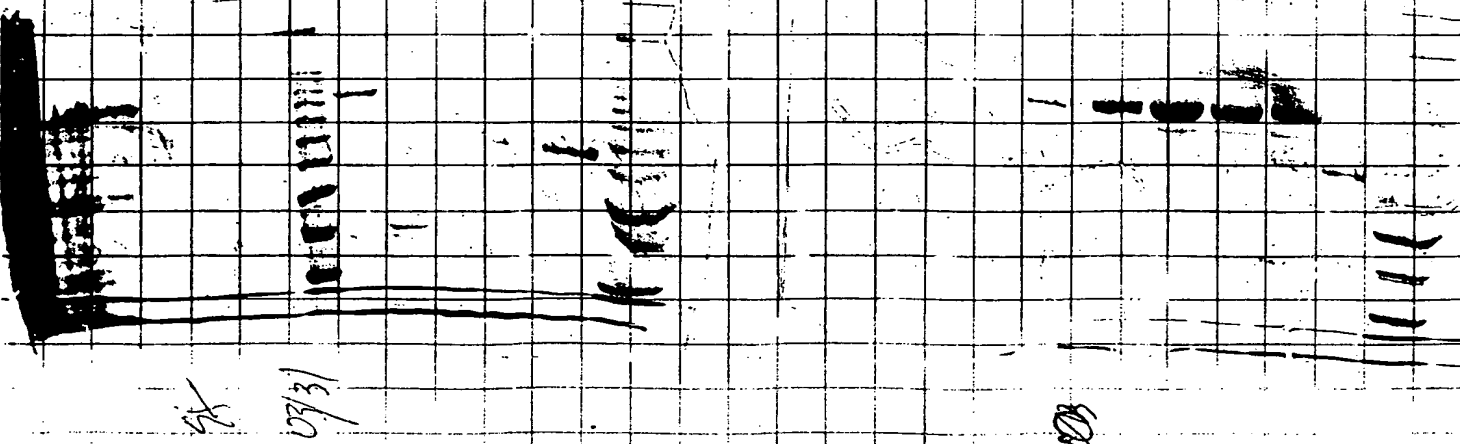
27 23/31

PAGE -

|      |      |     |      |   |      |    |    |    |    |
|------|------|-----|------|---|------|----|----|----|----|
| 1    | 2    | 3   | 4    | 5 | 6    | 7  | 8  | 9  | 10 |
| Cude | Heat | PEI | AS   | M | Load | 17 | 18 | 19 | 20 |
|      | Kill |     | Sup. |   |      |    |    |    | M  |

|    |    |    |    |    |    |    |    |    |    |
|----|----|----|----|----|----|----|----|----|----|
| 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 |
| 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 |
| 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 |

27 23/31



To Page No. \_\_\_\_\_

sed & Understood by m ,

Mary Forre

Dat

4/15/95

Invented by

E. Kym

Recorded by

Date

23/31/95

TNE

Page N. \_\_\_\_\_

Goal: To clone the TNE 35 Fy (mut) into pTTQ19 or a similar vector.

New Scheme: pUCTNE 35 Fy (2.5 kb) → H3 → Klenow → SphI → SclI/puc19  
2 kb  
SclI

Clone into the  
SmaI/SphI site of pTTQ19.

|                  |    |        |
|------------------|----|--------|
| pTTQ19           | 4  | = ~2.5 |
| 10xR2            | 4  |        |
| H <sub>2</sub> O | 30 |        |
| SmaI             | 2  |        |
| 100 µl           | 40 |        |

|                  |       |        |
|------------------|-------|--------|
| pUCTNE 35 Fy     | 20    | = ~1.5 |
| 10xR2            | 4     |        |
| H <sub>2</sub> O | 14    |        |
| H3               | 2     |        |
| 100 µl           | 40 µl |        |

30° c - 1 hr.  
8/3/95

8/3/95

- 1 pUCTNE 35 Fy cut with H3
- 2 pTTQ19 cut with SmaI

Cuts look good

|               |    |
|---------------|----|
| pTTQ19/SmaI   | 40 |
| SphI          | 2  |
| 100 µl        | 42 |
| 37° c - 1 hr. |    |

|                 |       |
|-----------------|-------|
| pUCTNE 35 Fy/H3 | 40.1  |
| 10xR2           | 10.1  |
| 10 mM dNTP mix  | 2.1   |
| Klenow          | 0.5.1 |
|                 | 52.5  |

ice 5'  
EDTA to 20 mM  
phenol extract  
TA 0.02 M

ed & Understood by me,

iburk

Date

8/3/95

Invented by

Recorded by

May Longo

Date

7/31/95

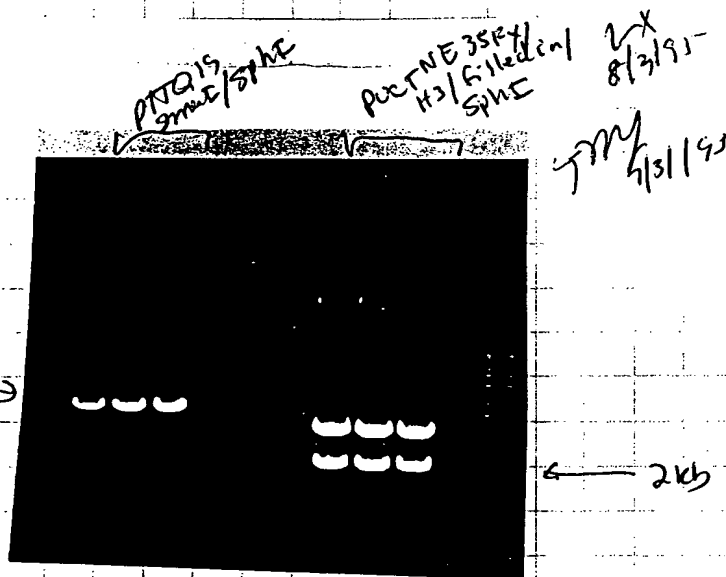
Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE TNE

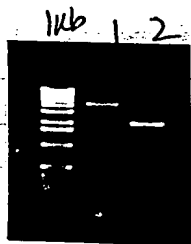
From Page No. \_\_\_\_\_

pUCTNE35Fy/H3 / filled in  $\rightarrow$  resuspended in 40  $\mu$ l 1X R6  
 2  $\mu$ l of 100  $\mu$ l SphI  
 37 $^{\circ}$ C - 1 hr.  
 applied to a 0.9% agarose gel.  
 Gel run at 180V.



cut bands out +  
 freeze at -20 $^{\circ}$ C.

GeneClean the frag as usual.  
 Dissolved in 10  $\mu$ l TE.  
 Applied 1  $\mu$ l to a 0.9% agarose gel.  
 Gel run at 180V.



- 1 pTA15 / smc2 / sphI
- 2 2 kb H3 / filled in / sphI frag  
 from pUCTNE35Fy

$\sim 10$  ng/ $\mu$ l = . .  
 $\sim 20$  ng/ $\mu$ l = . .

To Page N

With ss d &amp; Understood by me,

Lidunyan

Date

8/8/95

Invent d by

Recorded by

Lidunyan

Date

8/1/95

TNE

Project N \_\_\_\_\_

B k No. \_\_\_\_\_

183

ag N \_\_\_\_\_

lig

TQ19 / SmaI / SphI .003 pmol/.1  
 16 H3 / Filled in / SphI .015 pmol/.1  
 5X ligase buffer  
 H<sub>2</sub>O  
 Ligase (10)

|      |
|------|
| 2    |
| 1.5  |
| 1    |
| 4    |
| 12.5 |
| 1    |
| 20.1 |

RT - 30 min.

Jason xformed 2ul of the lig with 100ul DH10B CC.  
 std xform. Plated 10% + 90% on yet amp plates. 37°C ON

#2      10%      90%  
          18      ~150

picked 8 colonies into 3 mls of CG + amp<sup>100</sup>. 37°C - ON.

mp as usual. Dissolved in 5ul TE.

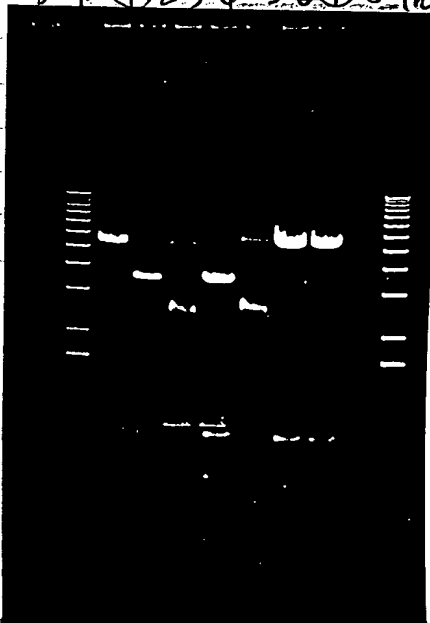
mp      3  
 IDRL      2  
 H<sub>2</sub>O      13  
 SphI      1  
 EcoRI      1  
          10

37°C - 1hr.

Applied to a  
 0.5% agarose  
 gel. Gel  
 run at 100V

sub PUCTNE 35 PY mot into  
 SmaI / SphI site of pTQ19  
 clones cut E SmaI / EcoRI  
 5 kb 1 2 3 4 5 6 7 8 9 10

ANY 8/1/95



To Page No. \_\_\_\_\_

sed &amp; Understood by m ,

Date

Invented by

Date

Lisha Xu

8/3/95

R corded by

CONY Longo

2/13/95

58

Project No. 20221

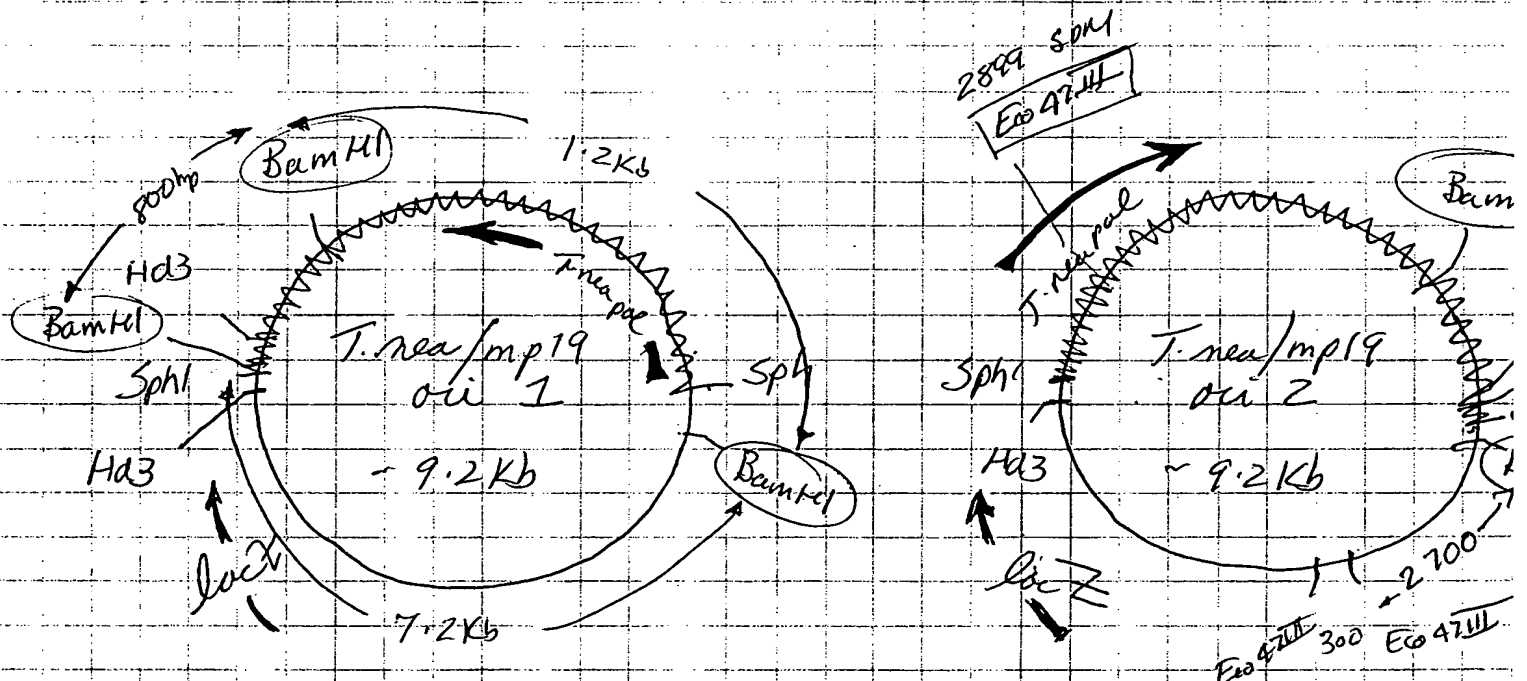
Book No. 3884

TITLE *T. neapolitana* SDM

From Page No. 57

February 8, 1985

- I added 200  $\mu$ l of DH5 $\alpha$  F' IQ lawn cells to 10 ml. circle brown.
- I added 1 ml of the cells to 8 glass tubes
- Each tube was inoculated with a clear plug and incubated at 37°C (8:00 am →)



|       |        |          |        |            |
|-------|--------|----------|--------|------------|
| SphI  | 7.2 Kb | VECTOR   | 7.2 Kb | VECTOR     |
|       | 2 Kb   | INSERT   | 2 Kb   | INSERT     |
| BamHI | 7.2 Kb | VECTOR   | 8.4 Kb | VECTOR + I |
|       | 1.2 Kb | } INSERT | 0.8 Kb | INSERT     |
|       | 0.8 Kb |          |        |            |

Add *T. nea*/pSPORT as a positive control for both digests

Witnessed & Understood by me,

May Longo

Date

2/14/85

Invent d by

Recorded by

Robert Schmidt

Date

2-8-85

To Page

ag No 58

February 8, 1995 (Wednesday)

## DIRECT SCHEMES

|                   | PER RXN  | x | 9 = | COCKTAIL |                                     |
|-------------------|----------|---|-----|----------|-------------------------------------|
| 1. HOH            | 7 $\mu$  | x | 9 = | 63 $\mu$ | <input checked="" type="checkbox"/> |
| React 6) 10x Bfr  | 2        | x | 9 = | 18 $\mu$ | <input checked="" type="checkbox"/> |
| DNA               | 10       |   |     |          |                                     |
| (100 $\mu$ ) SpH1 | 1        | x | 9 = | 9 $\mu$  | <input checked="" type="checkbox"/> |
| TOTM              | 20 $\mu$ |   |     | 90 $\mu$ |                                     |

For T-neap/SPORT  
control add

Tp E1 20  $\mu$  ☒  
 DNA 2  $\mu$  ☒  
 TOTM 22  $\mu$  ☒

✓

add 10  $\mu$  to reaction

|                    | PER RXN  | x | 9 = | COCKTAIL |                                     |
|--------------------|----------|---|-----|----------|-------------------------------------|
| HOH                | 7 $\mu$  | x | 9 = | 63 $\mu$ | <input checked="" type="checkbox"/> |
| React 3) 10x Bfr   | 2        | x | 9 = | 18       | <input checked="" type="checkbox"/> |
| DNA                | 10       |   |     |          |                                     |
| (100 $\mu$ ) BamHI | 1        | x | 9 = | 9        | <input checked="" type="checkbox"/> |
| TOTM               | 20 $\mu$ |   |     | 90 $\mu$ |                                     |

Continued on page 1 of Notebook 3966

Aroni Patel

Gel photo

T Page No. \_\_\_\_\_

sed &amp; Und rstood by me,

Date

Invented by

Date

May Longo

2/11/95

Recorded by

Dion J. Leland

2-8-95

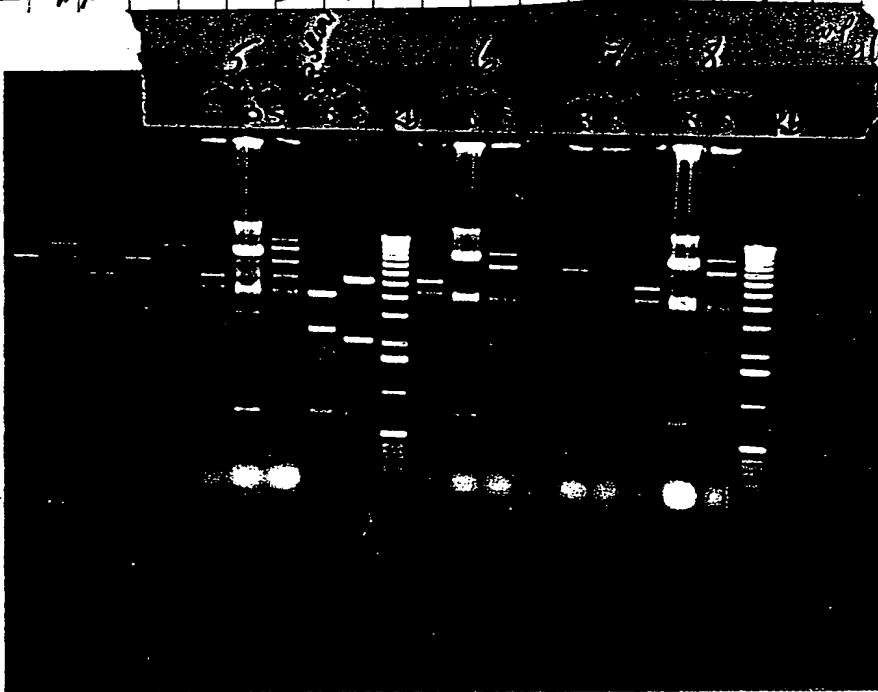
ig N \_\_\_\_\_

Con'd from 3884 NB

2/18/95 wed

MINIPREP DNA

- cfg 500  $\mu$ l of cells for 1 minute in an eppendorf cfg (centrifuge)
- removed supernatant and resuspended pellet in 100  $\mu$ l of 1X PEBI (SI) <sup>(saved)</sup>
- added 200  $\mu$ l of alkaline - SDS mix
- placed the tubes on ice for few minutes (3-5 min.)
- added 150  $\mu$ l of 7.5 M Ammonium Acetate
- Mixed the tubes by inverting
- cfg the tubes for  $\sim$  7-10 min.
- transferred 400  $\mu$ l supernatant to the new eppendorf tube
- added 800  $\mu$ l of ethanol to supernatant. Mixed tubes.
- incubated the tubes for  $\sim$  2 min. Spin.
- dissolved pellet in 50  $\mu$ l of TE + RNase A.
- applied 5  $\mu$ l to a 1% agarose gel.



SI = 0.9% glucose

25 mM Tris HCl (pH 8.00)

10 mM EDTA

alkaline - SDS mix = 1% SDS

0.1 N NaOH

To Page No. \_\_\_\_\_

Used &amp; Understood by me,

Date

Invented by

Date

Recorded by

Dwan

4/12/95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

70

From Page No. \_\_\_\_\_

Tf (min) /

1.33

4

Agarose

10 5 10 15 20 25 30 36 10 5 10 15 20 25 30 36 cycles

100-100,000

100-100,000



0/N 100-100,000 500-100,000

Result: no 13.5 kb product - maybe too much Mg<sup>+</sup> with hot primer

nonspecific smear seen with E+Br (P69) is cold in auto rad above

To Page N

Witnessed & Understood by me,

Date

Invented by

Date

Deena Golap

11/29/94

Recorded by

10-27-94

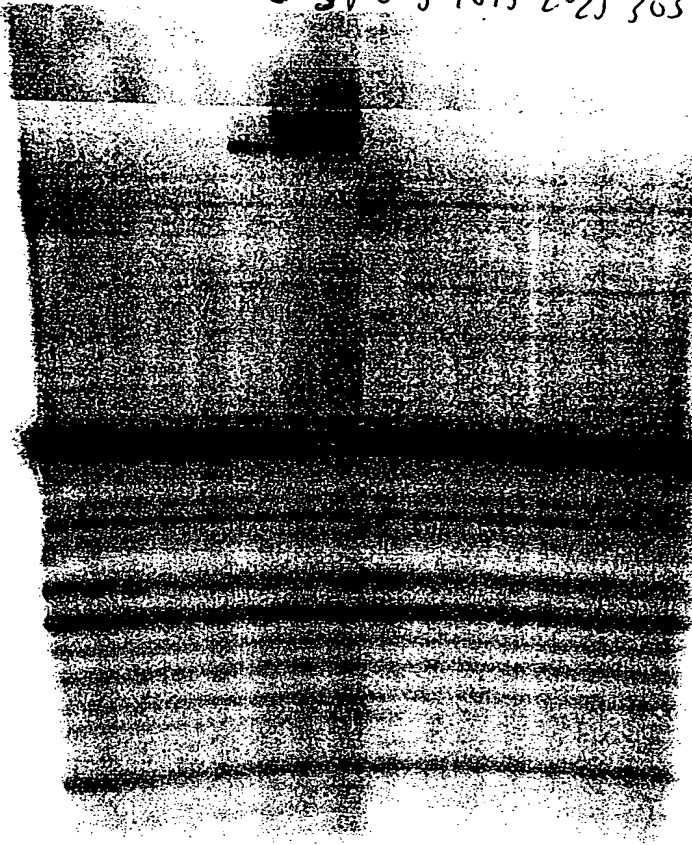
Tag N \_\_\_\_\_

PAGE

Tf1 units 1.33

4u

0 5 10 15 20 25 30 36 0 5 10 15 20 25 30 36 cycles

O/N  
experiment

100 -

100, 100

gray

Results  
only slight degradation ( $< 1$  nt/primer) after  
36 cycles  
note formation of primer dimer cycles 25-36  
but only for low Tf1!

To Page No. \_\_\_\_\_

Read &amp; Understood by m ,

Date

11/29/94

Invented by

Rec rd by

Date

10-27-94

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE Q LeSDM . Tosohakos .

14

rom Pag No. \_\_\_\_\_

Used DEPC treated water to make all buffers from now on out.

Washed column + column matrix extensively with  
5M NaOH -

Poured a 40 mL Q LeSDM - 8cm x 2.5cm - col  
Wash w/ 5M NaOH  
Wash w/ 1L of DEPC treated sterile H<sub>2</sub>O  
Wash & Equilibrate w/ Buffer A

Buffer A -

25mM KPO<sub>4</sub> pH 7.2  
10% glycerol  
10mM KCl  
5mM Bme  
1mM PMSF

Buffer B -

25mM KPO<sub>4</sub> pH 7.2  
10% glycerol  
800mM KCl  
5mM Bme  
1mM PMSF

↓  
abto conductivity 3mS

50mS

Sample conductivity 4mS - dialyzed in buffer A - ~ 27.5 mL - cor  
from dialysis

Program -

Load 0.5 mL/min.

Wash w/ 120 mL of Buffer A 1mL/min - collect 7.5 mL fractions 2.

Gradient - 400 mL linear gradient Buffer A - Buffer B - 1mL/min " 10

Wash w/ 120 mL of Buffer B 1mL/min collect 7.5 mL fractions -

To Page N

Witnessed & Understood by me,

May Longo

Date

4/5/95

Invented by

E. Hyman

R corded by

Date

4/2/95

Project No. \_\_\_\_\_  
B ok No. \_\_\_\_\_

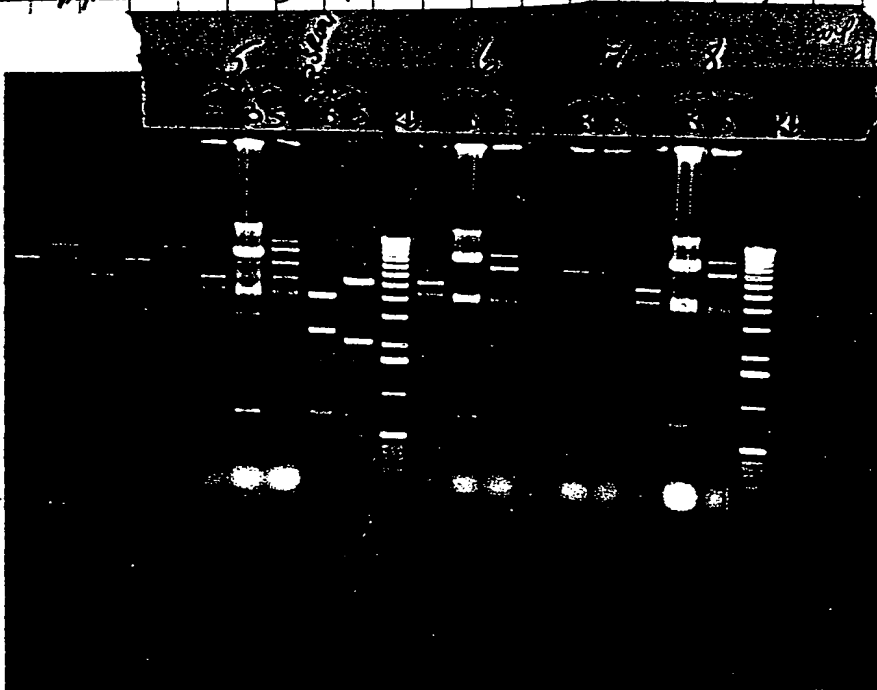
1

ig N. —

2/18/95 wed

Con'd from 3884 NB  
MINIPREP DNA

- cfg 500  $\mu$ l of cells for 1 minute in an eppendorf cfg (centrifuge)
- removed supernatant and resuspended pellet in 100  $\mu$ l of 1X PEBI (SI) <sup>(saved)</sup>
- added 200  $\mu$ l of alkaline - SDS mix
- placed the tubes on ice for few minutes (3-5 min.)
- added 150  $\mu$ l of 7.5 M Ammonium Acetate
- Mixed the tubes by inverting
- cfg the tubes for  $\sim$  7-10 min.
- transferred 400  $\mu$ l supernatant to the new eppendorf tube
- added 800  $\mu$ l of ethanol to supernatant. Mixed tubes.
- incubated the tubes for  $\sim$  2 min. Spin.
- dissolved pellet in 50  $\mu$ l of TE + RNase A.
- applied 5  $\mu$ l to a 1% agarose gel.



SI = 0.9% glucose  
25 mM Tris HCl (pH 8.00)  
10 mM EDTA

alkaline - SDS mix = 1% SDS  
0.1 N NaOH

To Page No. \_\_\_\_\_

ss d & Understood by me,

Date

Invented by

Dat

Recorded by

4/12/95

**From Page No.\_\_\_\_**

\* AAT II #2 : GTT TCT UAG ACG UCA GGU GGC ACC  
du 29 meu. TTT

**To Page No**

7/23/20

ag N. \_\_\_\_\_

primers old } = 20x : ~~2.5~~ 10 + 10 ml of each primer at (100 µl)  
 do }  
 1.80 ml 11/20

primers do } = } equivalent amount of each  
 new }  
 other 2 different combos } 50 + 50 ml (10 µl each)  
 1 do + other new do }

2 V: buffer 60  
 dNTP 12  
 01) Temp 2.4  
 me elongase 12.0 (1.01/µl)  
 H<sub>2</sub>O 398.6  
 480.0

2 V alone 0.5 µl 1 µl  
 buffer 60 60  
 dNTP 12 12  
 Temp 2.4 2.4  
 elongase 3.0 6.0  
 H<sub>2</sub>O 402.1 399.6

ube & (12 - 22)

(23 - 33)

(33 - 44)

11, 22, 33 & 44 w/o any primers.

cycling: 94°, 3'

30( 94°, 30", 50°, 30", 72°, 2' ) → 4° soak

samples thrown out 12/19/94

To Page No. \_\_\_\_\_

ssed & Understood by me,

*[Signature]*

Date

11/2/94

Invent d by

R corded by

K. Sitarman

Dat

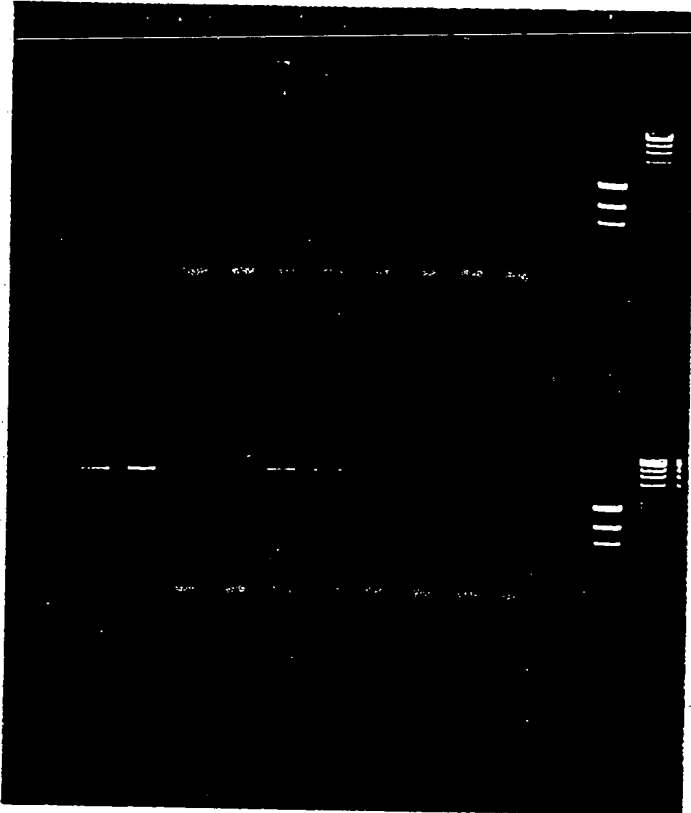
11/23/94

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

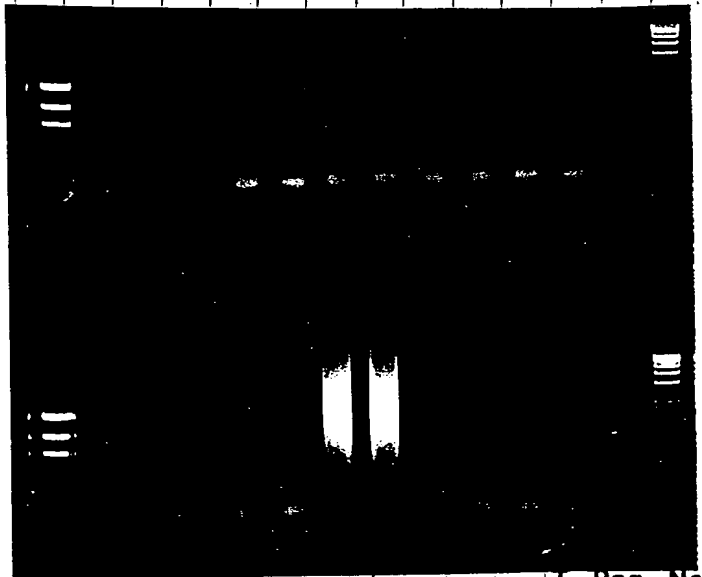
m Page N \_\_\_\_\_

Tag:Result:

- Even with Tag problem with product at the annealing temp.
- F/R - no du product as slightly with Tag, more with T + D.V.
- old primer at 50°, did work with Tag.
- with Tag + D.V. misper all most gone with old primer new misper product with T + D.V.
- misper didn't work at all.

N + N + 0 + -/+ -/+ no primer

D.V. 0.50

Tag + D.V. 1 : 0.01 :Deep vent as usual  
didn't work →with old du primer smears  
for the first time.Why more primer dimer  
with du - than with  
non du?

ness d &amp; Understood by me,

Date

Inv nted by

Date

Record d by

Pag No

11/23/94

sk A.K. ...

11/23/94

Project No. \_\_\_\_\_  
B ok No. \_\_\_\_\_

183

TNE

ag N

lig

TQ19 / SmaI / SphI .003 pmol/.1  
kb H3 / Filled in / SphI .015 pmol/.1  
5X ligation buffer  
H<sub>2</sub>O  
Ligase (10)

2  
1.5  
1  
4  
12.5  
1  
20.1

RT - 30 min.

Jason xformed 2 ul of the lig with 100 ul DH10B cc.  
std xform. Plated 10% + 90% on pet amp plates. 37°C ON

#2 10% 90%  
18 ~150

picked 8 colonies into 3 mls of CG + amp 100. 37°C - ON

mp as usual. Dissolved in 50 ul TE

mp 3  
IDRL 2  
H<sub>2</sub>O 13  
BpH 1  
EcoRI 1  
10

sub PUL TNE 35 PY mut into  
SmaI / SphI site of pTTQ19  
clones cut E SmaI / EcoRI  
2 kb 1 2 3 4 5 6 7 8 1 kb

ANY 8/1/95



37°C - 1 hr.  
Applied to a  
0.9% agarose  
gel. Gel  
run at 100V

4875  
2000  
6575

To Page No. \_\_\_\_\_

sed & Understood by me,

Date

Inv nted by

Date

8/3/95

Recorded by

8/13/95

Lisha Xin

Colony Long

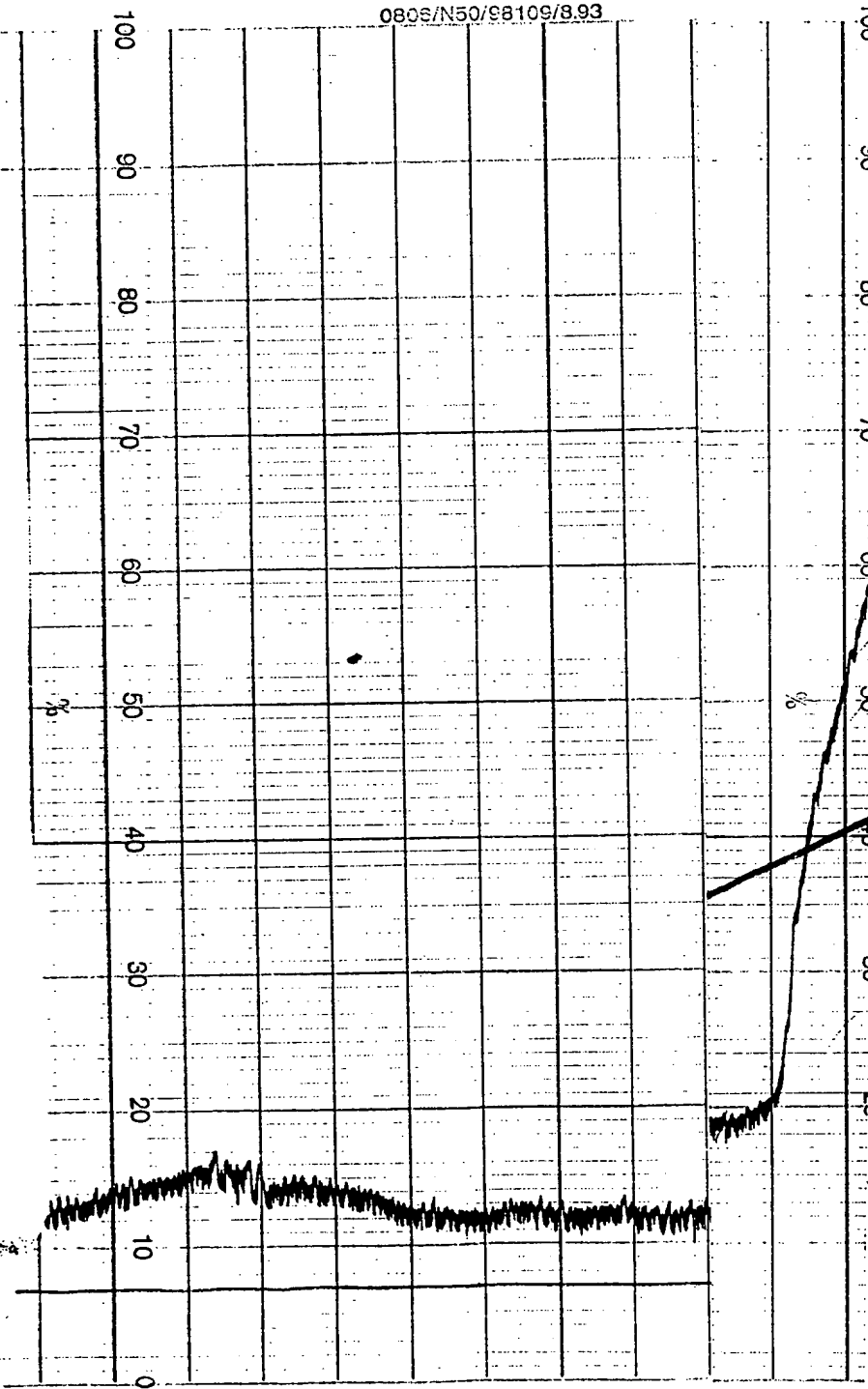
Project No. \_\_\_\_\_  
B k No. \_\_\_\_\_

115

Deparin Q 650 Tne-Durification

Page No. \_\_\_\_\_

0806/N50/98109/8.93



24 04/03/95

gmm 4/5/95

To Page No. \_\_\_\_\_

Read & Understood by me,

Date

Invented by

Date

May Longo

4/5/95

Recorded by

04-103/95

Project No. \_\_\_\_\_  
Book No. \_\_\_\_\_ TITLE \_\_\_\_\_

2

From Page No. \_\_\_\_\_

2/9/95 th

# Purification of m13 ssDNA

1. cfg 1.0 ml of \*infected cell culture for 2 min. (1 to 5 m)
2. Transferred 800.0  $\mu$ l to the new tubes  
(Pellet was saved for isolation of RF DNA)
3. cfg supernatant again to remove any residual cells
4. added 200.0  $\mu$ l of 20% PEG + 1.5 M NaCl. Vortexed
5. Incubated tubes at room temperature for 5 min.
6. cfg tubes for 5 min. & discarded supernatant (sup.)
7. added 200  $\mu$ l of \*TE & vortexed really good.
8. cfg for ~ 1-2 min. (to remove any residual cell debris)
9. transferred sup. to the new tubes. (RNaseA can be added here)
10. added equal vol. of Phenol / chloroform / isoamyl alcohol  
(25:24:1) Mixed well.
11. cfg 5 min.
12. removed the aq. (upper) layer to a new tube (be very careful)
13. added  $\frac{1}{10}$  vol. of 3M NaAc +  $2\frac{1}{2}$ -3 vol. of 95%.
14. Incubated @  $-70^{\circ}\text{C}$  till 2/14/95.

$\left\{ \begin{array}{l} 20.0 \mu\text{l NaCl} \\ 600.0 \mu\text{l ET} \end{array} \right.$

TE ( $T_{10}E_1$ ) = 10 mM Tris-HCl pH 8.0 + 1 mM EDTA pH 8.0

infected cell culture = <sup>①</sup>grew an E. coli  $F'$  strain to an OD of 0.4 in 2xYT  
 $\downarrow$   
 $F'$  = Fertility Factor: codes for tra genes & pilis to allow infection of the  
m13 Phage. (transfer of DNA)

Cont'd - - - To Page No

Witness d & Understood by me,

Date

Invented by

Date

Recorded by

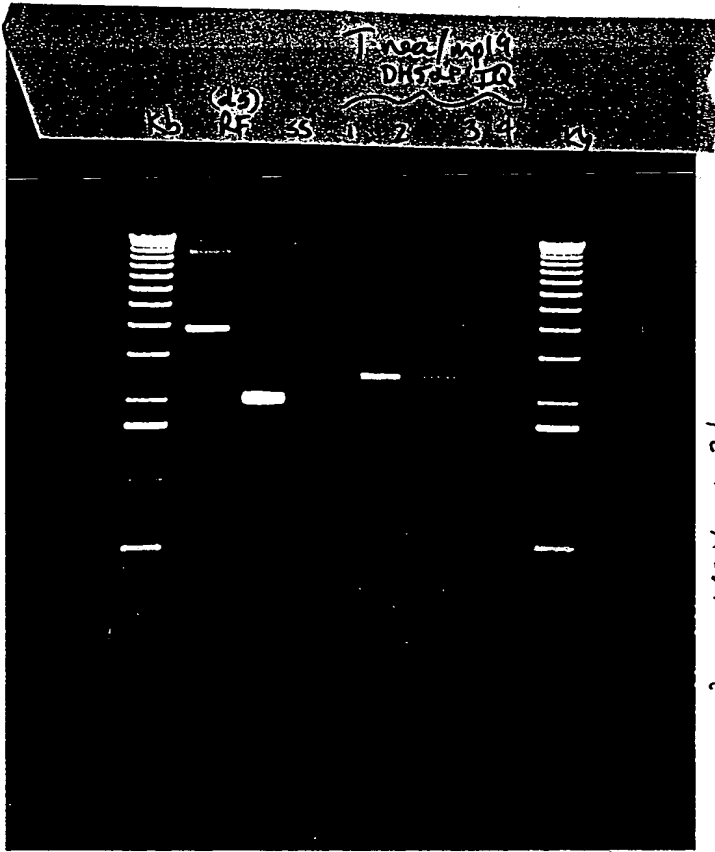
4/12/95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

5

Page No. \_\_\_\_\_



Run 140V ~ 2 hrs.

ap 4/12/95

To Page No. \_\_\_\_\_

Read & Understood by me,

*J. Polansky*

Date

4/12/95

Invented by

Recorded by *J. Polansky*

Date

4/12/95

From Page No. —

8/1/95

lig

PTTQ19 / SmaI / SphI .003 pmol/ul  
 2 kb HB / Filled in / SphI .015 pmol/ul  
 5X ligation buffer  
 H<sub>2</sub>O  
 Ligase (10)

|       |
|-------|
| 2     |
| 1.5   |
| 1     |
| 4     |
| 12.5  |
| 1     |
| 20 ul |

RT - 30 min.

Jason xformed 2 ul of the lig with 100 ul DH10B cc.  
 std xform. Plated 10% + 90% on yet amp plates.

8/2/95

#2

10%  
1890%  
~150

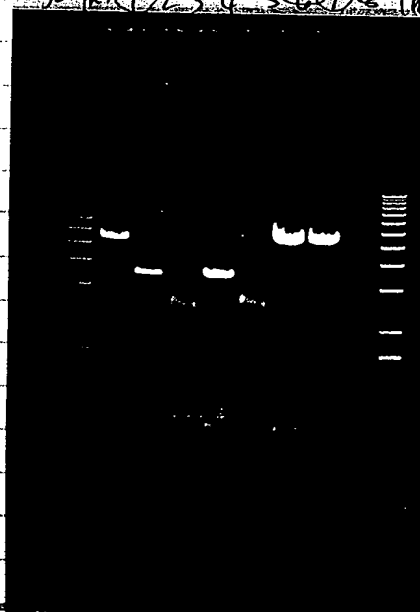
picked 8 colonies into 3 ml of CG + ampicillin. 37°C -

8/3/95

mp as usual. Dissolved in 50 ul TE.

mp 3  
 DRL 2  
 H<sub>2</sub>O 13  
 BpH 1  
 EcoRI 1  
 10

sub PUCTNE 35 PY mot into  
 SmaI / SphI site of PTTQ19  
 cloned site SphI / EcoRI  
 5 kb 2.3.4 5 kb 3.1 kb

ANY<sub>81</sub>

Vector 4575  
 insert 2000  
 6575

37°C - 1 hr.  
 Applied to a  
 0.8% agarose  
 gel. Gel  
 run at 180V

To Page No.

Witness d &amp; Und rstood by m ,

Lisa Xu

Dat

8/3/95

Invented by

Recorded by

C. M. L. L. L.

Date

8/13/95

annealing temperature

Project N \_\_\_\_\_

pmc9 / dif. emg / dif. primer / dif

Book No. \_\_\_\_\_

11/28/94

109

Tag No. \_\_\_\_\_

purpose: To check at different annealing temp to get rid of mispriming during pmc9 amplification

Temp checked 58°, 60°, 62°, 65°

56° gave more non specific bands than 58°. pg 102 -

checked all three primer set { Adul- II new dv  
 " " " - dv  
 " old dv 2728 + 29

Amplified with Tag, Tag + DV, DV alone.

200 µM dNTP  
 1 µM primer  
 200 µg template  
 2 mM Buffer (from buffer) - used Deep Vent buffer

prepared cocktail for 80 Rx

added primers separately, adding respective enzymes.

|        |         |           |        |                               |
|--------|---------|-----------|--------|-------------------------------|
| well # | 1 - 8   | Tag       | old dv | each combination              |
|        | 9 - 16  |           | New dv | in duplicate                  |
|        | 17 - 24 |           | " - dv | annealed at different         |
|        |         |           |        | temp 58, 60, 62, 65°          |
|        | 25 - 32 | Tag + DV  | old dv |                               |
|        | 33 - 40 |           | new dv | 92° 3'                        |
|        | 41 - 48 |           | " - dv |                               |
|        |         |           |        | 30 (94°, 30", X 30", 72°, 3') |
|        | 49 - 56 | Deep Vent | old dv |                               |
|        | 57 - 64 |           | new dv | 72° 10'                       |
|        | 65 - 72 |           | " - dv |                               |
|        |         | 15 U      |        | 4° 20 min.                    |

To Page No. \_\_\_\_\_

ss d & Understood by me,

*[Signature]*

Date  
 12/19/94

Invented by

Recorded by

*[Signature]*

Date

11/28/94

Project No. \_\_\_\_\_

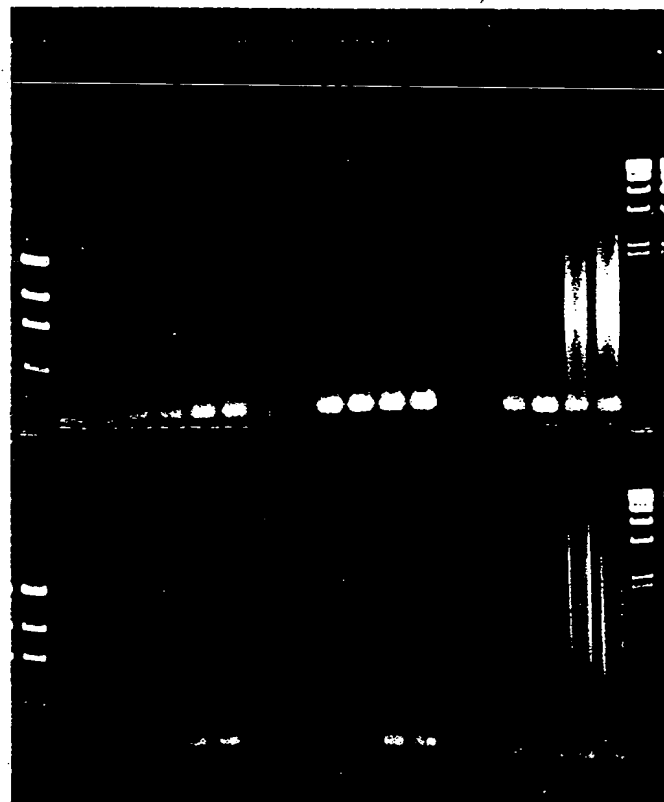
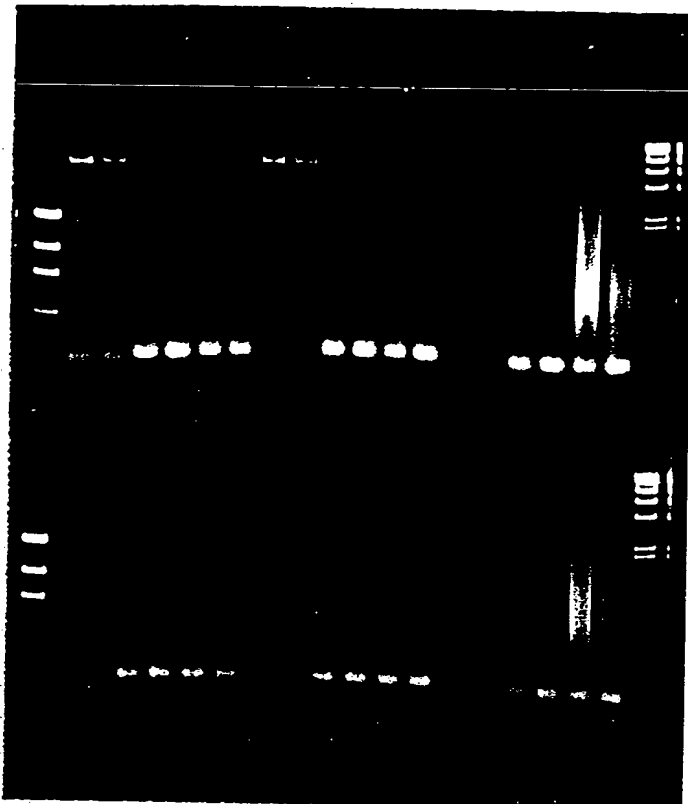
Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

110

From Page No. \_\_\_\_\_

Tag D.V. D.V. 58°  
 0-30 N. 40 TU

Result:

The only thing that worked,

Tag

Tag + D.V.

old dv primer at 58°

Deep Vent / non dv / at all anoxic temp remains?

All samples discarded

To Page N

Witnessed &amp; Understood by m,

Date

12/18/94

Invented by

Recorded by

sk. N. K. M. M. M. M.

Date

11/29/94

70

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

From Page N \_\_\_\_\_

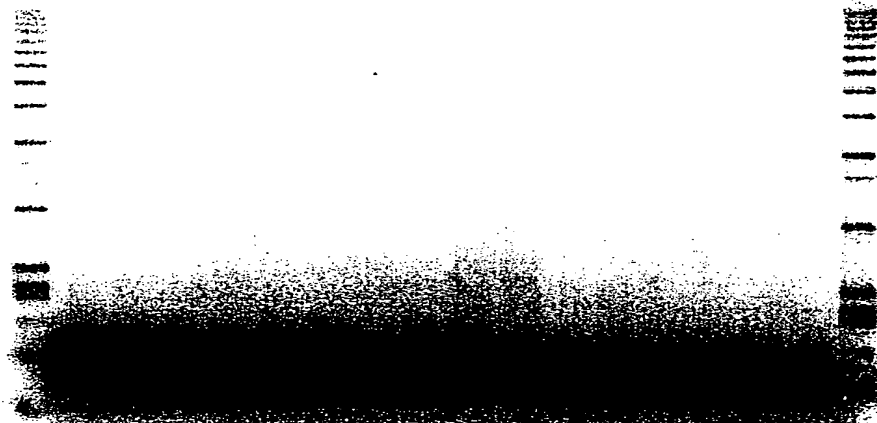
Tf (min)

1.33

4

Age

10 5 10 15 20 25 30 36 cycles



O/N spread 100 - 100,000 gray

Result: no 13.5 kb product - maybe too much Mg  
with hot primer!  
nonspecific smear ~~also~~ seen with E+Br (B69) is  
cold in auto rad ~~above~~ above

Witnessed & Understood by me,

Date

Invented by

Date

To Page

11/29/94

Recorded by

10-27-94

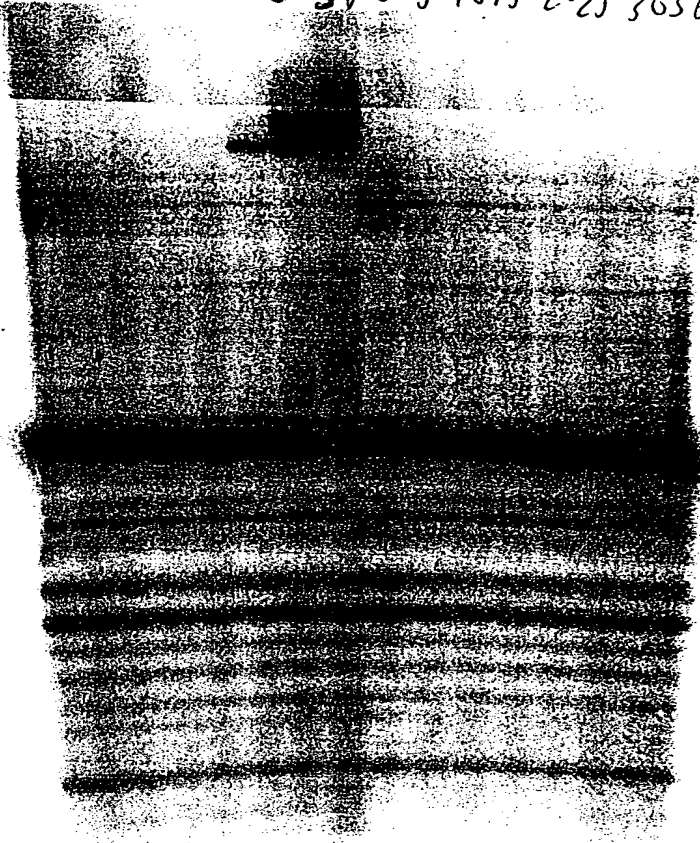
Deena Golay

PAGE

Tf1 rate 1.33

4u

0 5 10 15 20 25 30 36 0 5 10 15 20 25 30 36 cycles

O/N  
experiment

100 -

100, 100

gray

Result:  
only slight degradation ( $< 1$  nt/primer) after  
36 cycles  
note formation of primer dimer cycles 25-36  
but only for low Tf1!

T Page No. \_\_\_\_\_

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Date

Inv nt d by

Date

11/29/94

Record d by

10-27-94

74

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

From Page No. \_\_\_\_\_

make 2.5  $\mu$ l rTag (EXBT1 lot) by 1:1 dilution  
of 5  $\mu$ l (on P 61) with storage buffer:

5  $\mu$ l rTag P 61

20  $\mu$ l

storage buffer

20  $\mu$ l

VP 40  $\mu$ l

To Page N

Witnessed & Understood by me,

Deborah Pokrup

Date

11/29/94

Inv. nted by

Rec. rded by

Date

10-27-94

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE Units - on Loads + Pools -

118

From Page No. \_\_\_\_\_

Purpose: what to determine total units on Heparin +  
Q650 + the total units pooled -  
+ determine units / gram from crack sample

1. crude -  $\frac{1}{2000}$
2. after heat shock  $\frac{1}{2000}$
3. Load PET.
4. Load Hep  $\frac{1}{1000}$
5. Pool Hep  $\frac{1}{1000}$
6. Load Q650  $\frac{1}{1000}$
7. Pool (1) Q650  $\frac{1}{500}$
8. Pool (2) Q650  $\frac{1}{500}$

(7x3) = 21 samples -

Tag Dislution Buffer  
25mM Tris pH 8.0  
80mM KCl  
100  $\mu$ g/mL glycine  
1mM EDTA  
.5% NP-40  
.5% Tween 20  
1mM Bme

| SAM                 | CPM1     |              |
|---------------------|----------|--------------|
| 1 $\frac{1}{2000}$  | 1958.00  | Load Hep 118 |
| 2 $\frac{1}{2000}$  | 2486.00  | 42           |
| 3                   | 3196.00  | 48           |
| 4 $\frac{1}{1000}$  | 2746.00  | 49           |
| 5 $\frac{1}{1000}$  | 3998.00  | 7234         |
| 6                   | 5108.00  | 23           |
| 7 $\frac{1}{2000}$  | 3000.00  | 72           |
| 8 $\frac{1}{2000}$  | 4990.00  | 60           |
| 9                   | 5510.00  | 33           |
| 10 $\frac{1}{1000}$ | 4888.00  | 59           |
| 11 $\frac{1}{1000}$ | 7964.00  | 48           |
| 12                  | 8240.00  |              |
| 13                  | 7990.00  |              |
| 14                  | 10032.00 |              |
| 15                  | 8612.00  |              |
| 16                  | 428.00   |              |
| 17                  | 78186.00 |              |
| 18                  | 78040.00 |              |
| 19                  | 79558.00 |              |
| 20                  | 22.00    |              |
| 21                  | 26.00    |              |

78594.7  
SA = 49.7

# Not too good need to rean

4/5/95

To Page N

Witness d & Understood by m ,

Date

Invent d by

Date

May Longo

4/5/95

Rec rd d by

04/04/95

Project No. \_\_\_\_\_

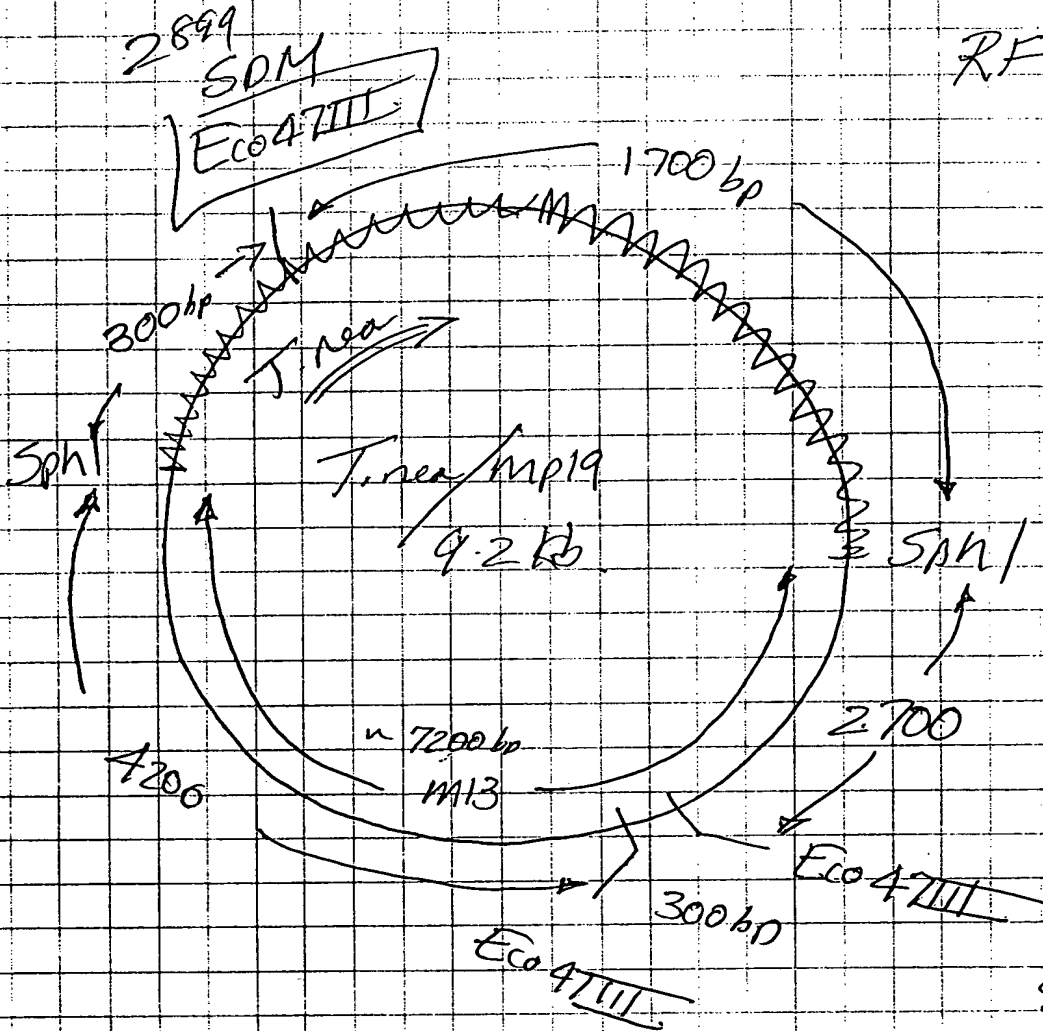
Book No. \_\_\_\_\_

61

Page No. \_\_\_\_\_

SDM 2899

RF map



See on file

|               | <u>Eco 47III</u>           |                                      | <u>1Kb</u> | <u>PARENT</u> | <u>MUTANT</u> |
|---------------|----------------------------|--------------------------------------|------------|---------------|---------------|
| <u>PARENT</u> | 8.9 Kb<br>0.3 Kb           | 5Kb<br>4Kb                           |            |               |               |
| <u>MUTANT</u> | 4.5 Kb<br>4.4 Kb<br>0.3 Kb | 3Kb<br>2Kb<br>1.6Kb<br>11Kb<br>500bp |            |               |               |

may be too light to see

|   |                     |                                  |                     |
|---|---------------------|----------------------------------|---------------------|
| Read & Understood by me,<br><br>May Jorgo | Date<br><br>2/16/95 | Invented by<br><br>D. J. Schmidt | Date<br><br>2-16-95 |
|   |                     | Recorded by<br><br>D. J. Schmidt |                     |

Page No. \_\_\_\_\_

The samples from previous expt were run on new Tablets  
received from Jim Spencer. (11/28/94) agarose.

1 Tab = 1 gm

Dissolved 2 gm in 200 ml of 1X TAE (0.2 mM EDTA)

Began to dissolve in few minutes at RT in buffer,  
looked like powder agarose (regular) in buffer.

microwaved for 4-5' (3' didn't completely go into soln)

added 5 µl of 10 mg/ml ethidium bromide

easy to pour, no bubbles } like ThermoGel - looked  
not dense } more like regular agarose

when solidified looked a bit transparent than regular  
agarose, well formed wells.

The gels were even at 100-105 V constant, along with  
11x14

DNA mass ladder and Hind III / Lambda.

Looked like it ran a bit faster than regular agarose.

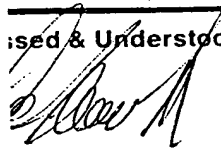
Ladders resolved quite well, the intensity of bands  
in mass ladder looked normal.

Apart from convenience of no need to ~~se~~ weigh out,  
there is no other added advantage.

maybe this gel is slightly faster than regular agarose  
so it really is 1%?

To Page No. \_\_\_\_\_

Used &amp; Understood by me,



Date

12/9/94

Invented by

Recorded by

K. Sitararaman

Date

11/29/94

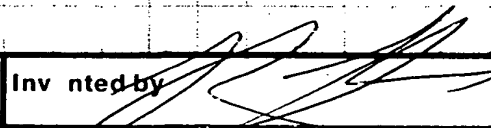
Same as  
 P/28, 6

54P primer for Vent digestion  
 and 25 ml on ribo ends

| ag       | N             |    |     |     |  |     |  |     |                             |
|----------|---------------|----|-----|-----|--|-----|--|-----|-----------------------------|
| mer      | 0.66 $\mu$ M  | ✓✓ | 8.1 |     |  |     |  | ✓   | 23 min. Work is 0.66 pm/min |
| (5 ng/l) |               |    |     |     |  |     |  |     |                             |
| 7.1      | 14.6 $\mu$ M  |    |     |     |  |     |  | ✓   | - -                         |
| 6.6 in   | = 1 $\mu$ M   |    |     | 6.1 |  |     |  |     |                             |
| 7.2      | 10.9 $\mu$ M  |    |     |     |  |     |  | ✓   | - r                         |
| 5.9      | = 1 $\mu$ M   |    |     | 6.1 |  |     |  |     |                             |
| 7.6      | 38.6 $\mu$ M  |    |     |     |  |     |  | ✓   | S -                         |
| 5.6      | = 1 $\mu$ M   |    |     | 6.1 |  |     |  |     |                             |
| 7.7      | 59.36 $\mu$ M |    |     |     |  |     |  | ✓   | S r                         |
| 59.36    | = 1 $\mu$ M   |    |     |     |  | 6.1 |  |     |                             |
| 10       | 11.6 $\mu$ M  |    |     |     |  |     |  | ✓   | - S                         |
| 1.8      | = 1 $\mu$ M   |    |     |     |  |     |  | 6.1 |                             |

Rinse buff ✓✓✓ 4 2 → ✓  
 3.4 PATP 3.4  $\mu$ M ✓✓ 4 2 → ✓  
 mCa/ml 11-4-94 ref ✓✓ 1 0.5 → ✓  
 PKK ✓✓ 2.9 → ✓  
 H<sub>2</sub>O ✓✓ 20  $\mu$ l 10.6 → ✓

37°C 30 min, 55°C, 5'

|  |                  |   |                |                  |
|--|------------------|---|----------------|------------------|
| s d & Understood by me,<br><br>maea Polamp | Date<br>11/29/94 | Inv nted by<br> | Dat<br>11-1-94 | To Pag No. _____ |
|  | Rec rd d by      |   |                |                  |

Project No. \_\_\_\_\_

B ok No. \_\_\_\_\_

119

SAM CPM1

|    |          |      |                       |
|----|----------|------|-----------------------|
| 1  | 4148.00  | 93   | Load Hepain           |
| 2  | 4852.00  | 55   |                       |
| 3  | 6730.00  | 40   |                       |
| 4  | 2580.00  | 42   | Post Hepain Load Q450 |
| 5  | 3952.00  | 34   |                       |
| 6  | 5700.00  | 25   |                       |
| 7  | 5318.00  | 31.7 | Pool Load Q450        |
| 8  | 3176.00  | 38   |                       |
| 9  | 2294.00  | 55   |                       |
| 10 | 3002.00  | 34   | Pool                  |
| 11 | 8568.00  | 33   |                       |
| 12 | 5524.00  |      |                       |
| 13 | 1742.00  |      |                       |
| 14 | 1812.00  |      |                       |
| 15 | 4872.00  |      |                       |
| 16 | 6352.00  |      |                       |
| 17 | 242.00   |      |                       |
| 18 | 82428.00 |      |                       |
| 19 | 81076.00 |      |                       |
| 20 | 77332.00 |      |                       |
| 21 |          |      |                       |

5.80 u/ml 4.75 positive control  
3.7

$\bar{x} = 80278$

SA = 800 cpm/nmol

Pooled together the two  
pools from Q450 - 16.5 mL  
added .5% w/v  
of TritonX + NP-40

Adams premix + 1.1 mL ~~water~~ <sup>ddCTP</sup> / 5

48 uL of g premix  
added to pre-labeled  
effluents - 1, 2, 4, 8  
of diluted sample  
was added - incubated  
for 10 minutes at  
74°C - the rxn was  
quenched w/ 10 uL  
g. 5 M EDTA +  
100 -

30 uL was spotted on  
6 FIC filters -  
TCA wash + EtOH wash  
dried & counted

alldr tubs made in serial

$$\left( \frac{10}{100} \right) \left( \frac{1}{x} \right)$$

x = 200, 150, 100,

|       | U/uL    | Total units         | vol.  | mg/mL | total mg | SA                 | /gram       |
|-------|---------|---------------------|-------|-------|----------|--------------------|-------------|
| AS1   | 62 u/uL | $1.3 \times 10^4$   |       | 1.4   | 30       |                    |             |
| load  |         | $1.3 \times 10^4$   | 21 mL | 1.4   | 30       | $4.3 \times 10^4$  |             |
| Pool  | 39 u/uL | $1.07 \times 10^4$  | 27.5  |       |          |                    | 77% recover |
| ad    | 39 u/uL | $9.675 \times 10^5$ | 25    | .323  | 8.0      | $1.22 \times 10^5$ | 3% pur.     |
| ol/   |         |                     |       |       |          |                    |             |
| algas | 38 u/uL | $6.27 \times 10^5$  | 16.5  |       |          |                    | 65% recover |

very conservative ~ 20,250 u/gram cell - for 100 mits - 500 gram crack

To Page No. \_\_\_\_\_

ssed & Underst od by me,

May Longo

Date

4/5/95

Invented by

Recorded by

Fig. 1

Date

04/05

4/15/85

Dat 04/05/2012

# QC. RNase Assay -

Project No. \_\_\_\_\_

Block No. \_\_\_\_\_

12

Page No. \_\_\_\_\_

| Tube | Rxn mix | Enzyme Unit | ul H <sub>2</sub> O |
|------|---------|-------------|---------------------|
| 1    | 50 ul   | 2           | 4 ul 8.5 ul         |
| 2    |         | 5           | 1 ul 3 4            |
| 3    |         | 10          | 2 ul 2 3            |
| 4    |         | 15          | 3 ul 2              |
| 5    |         | 20          | 4 ul 1              |
| 6    |         | 0           | 5 ul Dil'n Buffer   |
| 7    |         | 0           | 5 ul DEPC           |

Dilute Enzyme - 1/5

$$\frac{190 \text{ ul Tris}}{950 \text{ total volume}} = \frac{190}{(950-190)} = \frac{190}{760} \text{ dil'n Buffer}$$

$$\text{dilute to } 50 \text{ u/L} = \frac{1}{7.6} = \frac{10}{76} = \frac{10}{(76-10)} = \frac{10}{66} \text{ enzyme}$$

Rxn mix

Tag premix

PCR mix

10x Buffer A.G

8 ul

mRNA GluRin

160 ul 95% 320 ul 8 ul

processed H<sub>2</sub>O

232 ul 72 ul

400 ul

Incubate at 37°C in heat block for 1 hour -

Add

4 ug Proteinase K (2 mg/mL)

25 ug tRNA (5 mg/mL)

2 ul + 2.5 ul

Incubate 10 min @ 37°C

Add 20 ul 2 M NaAc + 200 ul 100% EtOH - vortex  
Keep in freezer - 20°C O/N

To Page No. \_\_\_\_\_

Read & Understood by me,

Date

Invented by

Date

Man Longo

4/5/95

Recorded by

4/5/95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

6

From Page No. \_\_\_\_\_

2/15/95 Wed.

(+) strand (ssDNA) lot # ED5702 260  $\mu$ g/ml  
RF strand (dsDNA) lot # CC 3111 5  $\mu$ g/18.4  $\mu$ l

calculation: ssDNA = 260  $\mu$ g/ml = ng/ $\mu$ l

$$\frac{260 \mu\text{g/ml}}{1000 \text{ ng}/\mu\text{g}} \cdot 1000 \text{ ng}/\mu\text{g} \cdot \text{ml} \cdot 1000 \mu\text{l} = 0.260 \mu\text{g}/\mu\text{l}$$

$$\frac{1000 \text{ ng}/\mu\text{g} \cdot (-.260 \mu\text{g})}{260 \text{ ng}/\mu\text{g}} = 260 \text{ ng}/\mu\text{l}$$

$$\frac{260 \text{ ng}/\mu\text{g}}{x \mu\text{g}} = 100 \text{ ng}$$

$$\left\{ \begin{array}{l} 260 \left( \frac{1}{2.6} \right) = 100 \text{ ng} \\ \text{or} \\ \frac{260}{2.6} = 100 \text{ ng} \end{array} \right.$$

for 2.6 total or final volume  
you need 1.0  $\mu$ l DNA

$$\begin{array}{r} 1 \mu\text{l DNA (260 ng}/\mu\text{l}) \\ 1.6 \mu\text{l TE} \\ \hline 2.6 \mu\text{l} \end{array}$$

for 100 ng/ $\mu$ l, } 2.0  $\mu$ l DNA (260 ng/ $\mu$ l)  
multiply by 2 } 3.2  $\mu$ l TE

dsDNA = 5  $\mu$ g/18.4  $\mu$ l.

$$1000 \text{ ng}/\mu\text{g} \times 5 \mu\text{g} = \frac{1000 \text{ ng}(5 \mu\text{g})}{\mu\text{g}} = 5000 \text{ ng}/18.4 \mu\text{l}$$

$$\frac{5000 \text{ ng}}{18.4 \mu\text{l}} = \frac{272 \text{ ng}/\mu\text{l}}{x \mu\text{l}} = 2.72 \text{ ng}$$

for 2.7 total volume you need  
1.0  $\mu$ l DNA

Total Volume (TV)

To Page 1

With ss d & Understood by me,

Date

Invent d by

Dat

4/12/95

R c rded by

4/12/95

Tag No. \_\_\_\_\_

|     | Tube # 1    |            |               | Tube # 2 |             |                        |
|-----|-------------|------------|---------------|----------|-------------|------------------------|
|     | RF (ds)     |            |               | ⊕ ssDNA  |             |                        |
| DNA | 1.0 $\mu$ l | $\times 3$ | $= 3.0 \mu$ l | DNA      | 1.0 $\mu$ l | $\times 3 = 3.0 \mu$ l |
| TE  | 1.7 $\mu$ l | $\times 3$ | $= 5.1 \mu$ l | TE       | 1.6 $\mu$ l | $\times 3 = 4.8 \mu$ l |
| TV  | 2.7 $\mu$ l | $\times 3$ | $= 8.1 \mu$ l | TV       | 2.6 $\mu$ l | $\times 3 = 7.8 \mu$ l |

Tube # 1, 2, 3, 4 of RF (dsDNA)

|                  | ①                                    | ②        | ③         | ④      |                                 |
|------------------|--------------------------------------|----------|-----------|--------|---------------------------------|
|                  | Alu I                                | Hind III | Sau 3 A I | Bam HI |                                 |
| H <sub>2</sub> O | 16.0 $\mu$ l                         |          |           |        | → (all 4 tubes w/ 16.0 $\mu$ l) |
| 10x Buffer       | 2.0 $\mu$ l                          |          |           |        |                                 |
| DNA              | 1.0 $\mu$ l                          |          |           |        |                                 |
|                  | (React 1; React 2; React 4; React 3) |          |           |        |                                 |
| Alu I            | +                                    | -        | -         | -      |                                 |
| Hind III         | -                                    | +        | -         | -      |                                 |
| Sau 3 A I        | -                                    | -        | +         | -      |                                 |
| Bam HI           | -                                    | -        | -         | +      |                                 |

Tube # 1, 2, 3, 4 of ⊕ (ssDNA) same order as RF

2 tubes were set-up for uncut, 1 with RF &amp; 2nd with ⊕

- each tube added 16.0  $\mu$ l H<sub>2</sub>O
- 2.0  $\mu$ l REact 2 10x buffer
- 1.0  $\mu$ l DNA

• Put all 10 tubes in

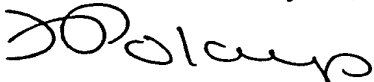
• ran the sample (all 10) on a gel next morning.

(0.8% agarose gel, 147 volts)

• picture of the gel is on the next pg (pg # 8)

To Page No. \_\_\_\_\_

Read &amp; Understood by me,

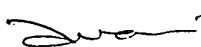


Date

4/12/95

Invented by

Recorded by



Date

4/12/95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_ TITLE \_\_\_\_\_

From Page No. \_\_\_\_\_

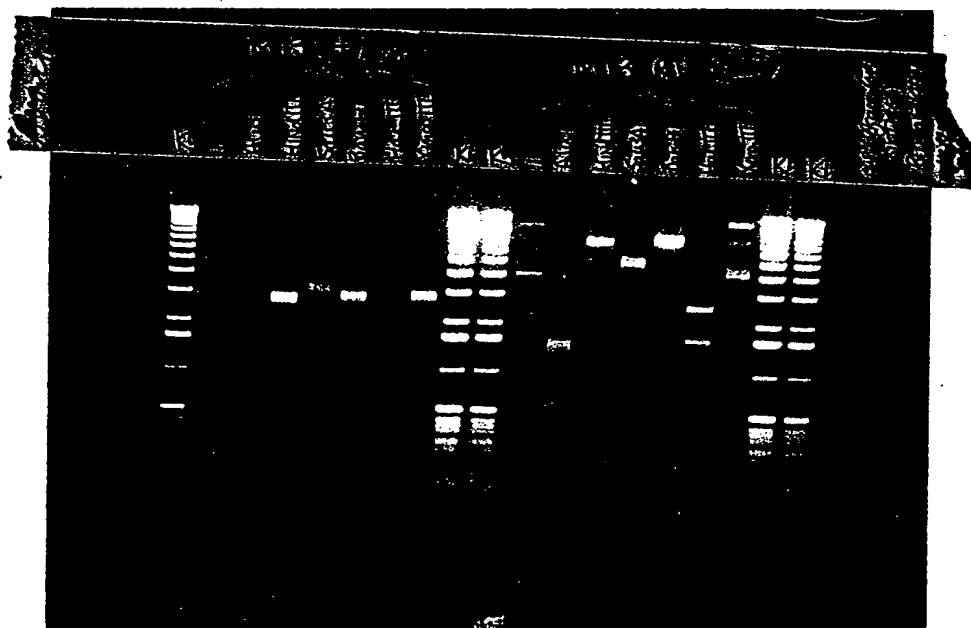
Tube# 1 T-neal / PTTG

1.0 ml

Tube# 2 T-neal / PTTG

1.0 ml

- Cfg. for 1 min. at room temperature
- discarded supernate and added: 100  $\mu$ l SI to the pellet. mixed  
200  $\mu$ l S2 lysis put both tub  
ice.
- Cfg for 5 min. at 4°C
- transferred 400  $\mu$ l of supernatant to the new tubes.
- added 800.0  $\mu$ l EtOH to the supernatant
- put both tubes in the fridge till tomorrow (2/16/95)



(+) Sall3A1 - gel shift (did not cut but binded)

To Page No. \_\_\_\_\_

Witnessed & Understood by me,

*[Signature]*

Date

4/12/95

Investigated by

Recorded by

*[Signature]*

Date

4/12/95

Project No. \_\_\_\_\_

112

Book No. \_\_\_\_\_ TITLE \_\_\_\_\_

From Page No. \_\_\_\_\_

Purpose: Since results are inconclusive -dV (new) (checked) gave more specific smear with Deepvent - previous (page 110) and gave the same type of smear with old + dV primers attempted to see whether this smear can be transformed into bands!

altered few conditions : checked 2 dif amounts of template

↓  
according to NCB suggestions:

1. reduce amount of Template
  2. " " of cycles
  3. increase Mg
  4. " dNTP
- 200 & 100 pg  
tried first 3.  
20 + 30 cycles  
2, 4 & 6 mM.

also included as controls were: Tag + (Tag + dV)

tried at 200 pg, 30 cycles, 200 μM dNTP with 2, 4, 6 mM

Two sets of reactions were made one with old dV & and the other with new - dV primers

Added Cocktail with different enzymes + later added more Mg accordingly. Used 10x Deepvent buffer has 2 mM Mg already

except for the Tag + (Tag + dV) control rest were run duplicates.

used 1:0.01 mix

5V/x

Deepvent used 1 unit / reaction

2V/x

94°, 3'  
20x (94°, 30" )  
30 (58°, 30" )  
72°, 3' /

To Page 1

Witnessed & Understood by me,

*[Signature]*

Date

12/16/94

Invented by

Recorded by

Date

11/30/94

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

113

Page No. \_\_\_\_\_

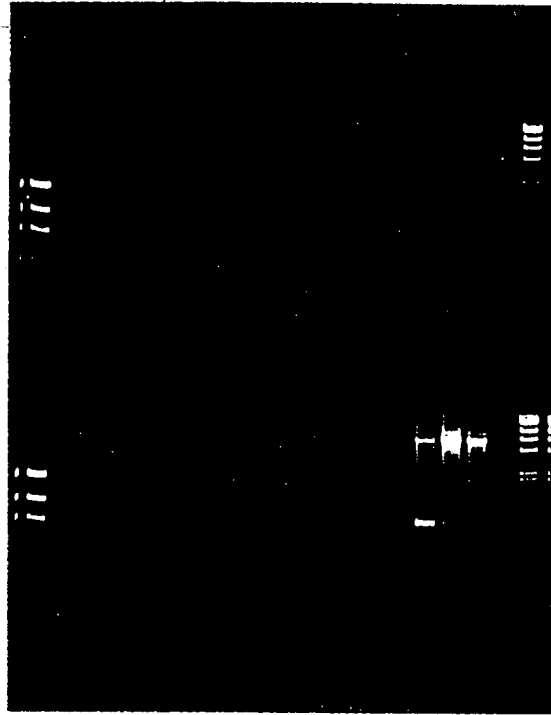
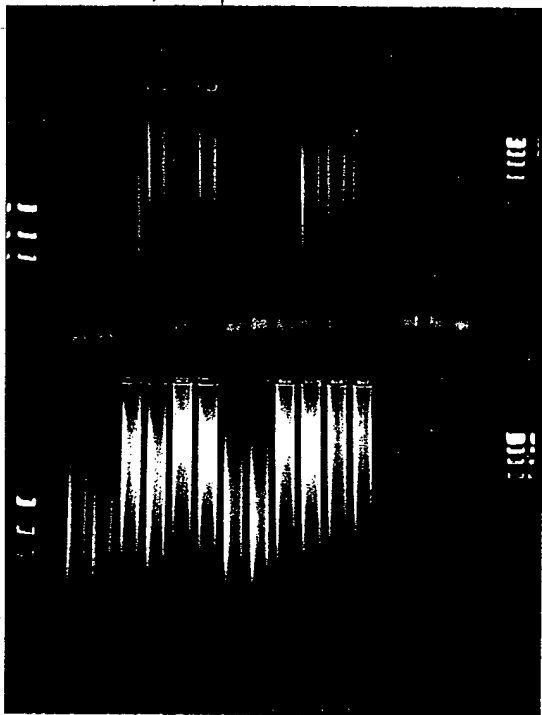
*new* *new* *dc* *primer* *Act II* *old* *dc* *primer* *2725* & *2729*

Deep Vent

Tag

Deep Vent

Tag  
642



25  
cy

30  
cy

Pg 200

100

642 *new*

200

100

2 4 1 2 4 6 Tag + DV

with Deep Vent  
these primers  
give readers

nothing worked

Consistent

- with Tag + DV  
works  
- w. Tag alone  
it doesn't

with Tag alone and  
Tag + Deep Vent  
nothing not even  
readers! would  
reproduce this

at 50° annealing

New primers are no good.

To Page No. \_\_\_\_\_

Read & Understood by me,

*[Signature]*

Date

12/18/84

Invented by

Recorded by

ck. Stannan

Date

11/30/84

76

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

Digestion of 5'P Z3mer by Vent  
± Tfl, Cheng vs Vent buffer.

From Page No. \_\_\_\_\_

3'P Z3 mer (P75)

(1) (2) (3) (4) (5) (6) (7) (8) (9)

3 3 3 3

Also with  
ribo a  
5' mer

2'P Z391

3

P 2692

3

P 2696

3

P 2698

3

P 2700

3

10x Vent buffer

10 10

10

X

✓

5x 67M Cheng

20 20

X

Mig (OAc) 12mm

7.5 7.5

X

0.9ml  
+ 0.3ml  
(f=1.2)

Vent 0.1u/l

1

Tfl 1u/l

1.24

1.24

H<sub>2</sub>O

86

85

68.5

70

86

X

✓

100 µl

70°C

remove 10 µl to 5 µl appt. seq. stop solution at

2, 5, 10, 20, 60 for (1)-(4)

(will 1 is Z3mer 0 time)

and

0, 3, 10, 60 for (5)-(9)

(take 0 point before pol add)

Program 143 = 70°C ∞

To Page No

Witnessed &amp; Understood by me,

Deena Polansky

Date

11/24/94

Invented by

Date

11-2-94

Recorded by

Project No. \_\_\_\_\_  
Book No. \_\_\_\_\_

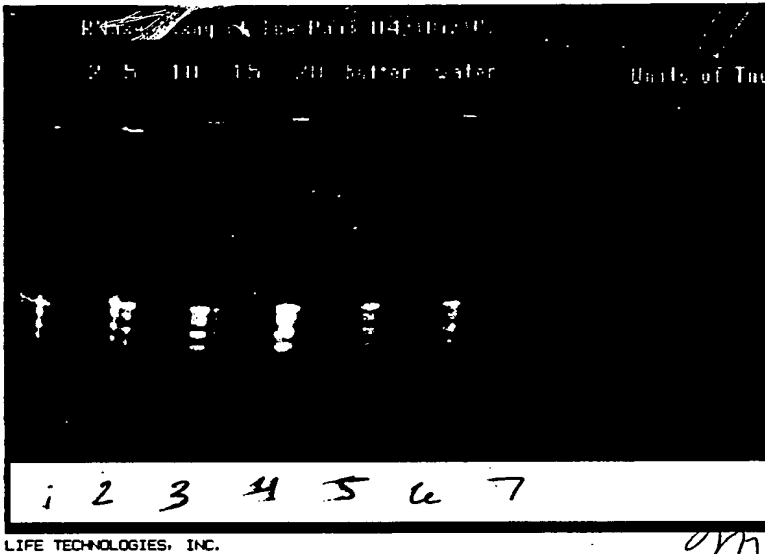
<sup>6</sup> j.m. 10  
TITLE Completion of RNase Assay -

From Page No. \_\_\_\_\_

Take samples from -20°C freezer - spin in micro centrifuge  
15 minutes -

decant etOH - air dry pellets -  
Add - m of RNA blue juice - heat 30 sec at 90°C  
Run out on 7.6%  
sequencing gel -

400 volts -

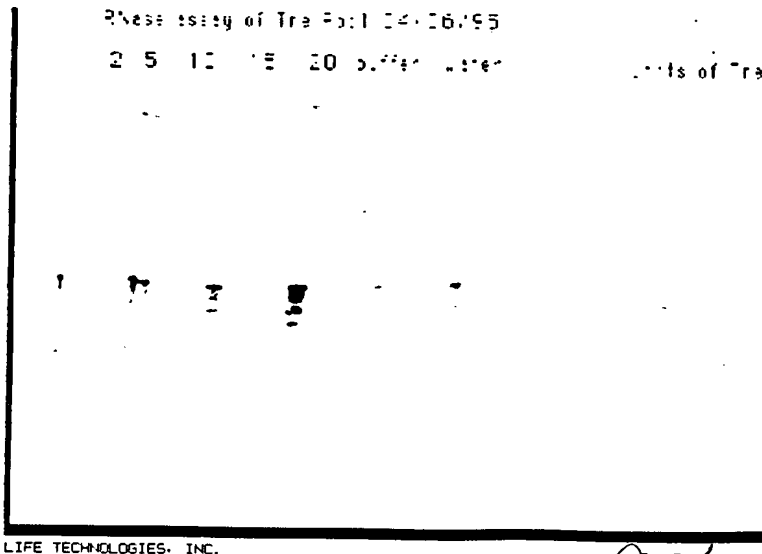


24-04/10/95

Conclusion -  
Appears to be  
RNase free! Next  
time use more RNase -  
Only used half of  
recommended amount  
used 1ug v- recommended  
2ug.

Bradford on Pools

24-04/10/95



24-04/10/95

24-04/10/95

To Page N

Witnessed & Understood by me,

May Longo

Date.

4/13/95

Invented by

E. Flynn

Recorded by

Date

04/06/95

# Exonuclease Assay - Tm Pool

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

123

Page No. \_\_\_\_\_

c. NO. 30042 SOP.

| Tube | Rxn mix<br>4.5 | Enzyme Units | $\mu$ | H <sub>2</sub> O       |
|------|----------------|--------------|-------|------------------------|
| 1    | ↓              | 0            | -     | 5 $\mu$                |
| 2    |                | 2.0          | ←     | 4 $\mu$ 50 $\mu$ l     |
| 3    |                | 5            | 1     |                        |
| 4    |                | 10           | 2     |                        |
| 5    |                | 15           | 3     |                        |
| 6    |                | 20           | 4     |                        |
| 7    |                | 0            |       | 5 $\mu$ l dil'n buffer |

| Rxn Mix                | 16 rxns -                          |
|------------------------|------------------------------------|
| 10x PCR                | 80                                 |
| 50mM MgCl <sub>2</sub> | 80                                 |
| S' ds sub              | 16 pmol 32 $\mu$ l 5 pmol/ $\mu$ l |
| B' ds sub              | 16 pmol 32 $\mu$ l 5 pmol/ $\mu$ l |
| H <sub>2</sub> O       | 494                                |
|                        | 720                                |

Heat @ 37°C for 1 hour - 1-7

Heat @ 72°C for 1 hour - 8-14

see page - 124 for data

To Page No. 124

Used & Understood by me,

Date

Invent d by

Date

Man tonge

4/15/95

R c rded by

04/06/95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE Endo Assay - 18038 QCP-T

From Page No. \_\_\_\_\_

Rxn Mixture - in 8 rxns -

(all tubes once before use -)

15264-013  
 .34 µg/µl  
 10x PCR buffer - 40 µl ✓  
 50 mM MgCl<sub>2</sub> - 40 µl ✓  
 → ΦX174 (± DNA) - 8 µg (23.5 µl) ✓  
 Autoclaved H<sub>2</sub>O 256.5

360 µl

Endo mix

H<sub>2</sub>O

Diluted enzyme 50/u

|   | Endo mix | H <sub>2</sub> O | Diluted enzyme 50/u |
|---|----------|------------------|---------------------|
| 1 | 45       | 5                |                     |
| 2 | 45       | 1                | 2 units - 2 µl      |
| 3 | 45       | 4                | 5 units - 1 µl      |
| 4 | 45       | 3                | 10 units 2 µl       |
| 5 | 45       | 2                | 15 units 3 µl       |
| 6 | 45       | 1                | 20 units 4 µl       |
| 7 | 45       | 5 Dil'n Buffer ✓ |                     |

Incubate @ 72°C in 3 hours -

90F

37°C

5.5 hours

Tag

Double Strand. Assay -

25260-027  
 EF 102 1702  
 .33 µg/µl  
 10x PCR buffer 40 ✓  
 50 mM MgCl<sub>2</sub> 40 ✓  
 - ΦX174 RF 8 24.2 ✓  
 Autoclaved H<sub>2</sub>O 255.8

360 -

Endo

H<sub>2</sub>O

Di. Enzyme 50/u

|   | Endo | H <sub>2</sub> O | Di. Enzyme 50/u |
|---|------|------------------|-----------------|
| 1 | 45   | 5                |                 |
| 2 | 45   | 1                | 2 4 µl 50/u     |
| 3 | 45   | 4                | 5 1 µl          |
| 4 | 45   | 3                | 10 2 µl         |
| 5 | 45   | 2                | 15 3 µl         |
| 6 | 45   | 1                | 20 4 µl         |
| 7 | 45   | 5 Dil'n buffer ✓ |                 |

To Page 1

With ss d &amp; Understood by me,

Date

Invented by

Dat

Manny Longo

4/13/95

R cord d by

04-06-95

# Endo Assay -

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

125

Page N \_\_\_\_\_

Spin samples down add 5  $\mu$ l of Blue Juice -  
Run out on 1.2% Agarose gel -

1 2 3 4 5 6 7 8 9 10 11 12 13 14



1 2 3 4 5 6 7 8 9 10

H<sub>2</sub>O 2 5 10 15 20 B

8 9 10 11 12 13 14

H<sub>2</sub>O 2 5 10 15 20 B

C = 4.0  
1.0  
6.0

at 10u -

4.5  
1.0  
4.5

conv  
5.1%  
10.1%

SS-Endo

DS-Endo

Endo looks good - however DS Endo - shows conversion to linear but this is also present in the buffer only lane - could just be a contaminant in the Dil'n buffer -

Dil'n Buffer used - from A.G. flasks from the 4°C beer cooler - orange tip -

Conclusion: - free of SS Endo nuclease - possible <sup>some</sup> DS endo nuclease but control w/tn buffer only shows significant conversion to linear so believe this is the dil'n buffer in or has <sup>DS endo</sup> activity not the enzyme prep.

To Page No. \_\_\_\_\_

Assessed & Understood by m ,

Date

Inv nted by

Date

Recorded by

Mary Tonga

4/13/95

S. Figure

5/10/95

Project N \_\_\_\_\_  
B ok N \_\_\_\_\_

23

The mutant Phe to Ala

ge No. \_\_\_\_\_  
① The same phenyl alanine corresponding to Tag polymerase  
will be changed to tyrosine

② For exo D will be changed to Alanine (corresponding  
region of Tag).

Brian cloned the SphI fragment of Tne Pol into M13mp.

I isolated the single stranded DNA from CJ238 as  
described before in Bio rad manual.

Test 5µl ssDNA

The DNA looks real good.

For D-A (3'-5' exo mutant oligo) is  
5' GA | CGT | TTC | AAG | CGC | TAG | GGC | AAA | AGA # 2899  
EcoRI site

For Phe → Tyr (O-helix) HpaI.  
~~AAA~~ GTA | TAT | TAT | AGA | GTA | GTT | AAC | CAT | CTT | TCC | A  
# 2904.

kinased 2899 before.

kinased 2904 as follows:  
2µl oligo (210 pmol)  
6µl 5X buffer (350mM Tris pH 7.6, 50mM MgCl<sub>2</sub>,  
50mM KCl, 5mM PMS)

1µl 10mM ATP  
0.5µl T4 Kinase (5U)  
20.5µl H<sub>2</sub>O  
5' at 37°C → Heat at 65°C  
+ 3µl TE  
To Page 24

sed & Understood by m ,

*[Signature]*

Date

4/8/95

Invented by

Recorded by

*[Signature]*

Date

3/14/95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

114

11/30/94

From Page No. \_\_\_\_\_

Prayer: Amplify GAPDH for ~~analysis~~ - cloning and ~~different~~ enzymes again.

- GAPDH / Regular Forward & Reverse primers / DeepVents worked (pg 97)
- never tried with Tag + DeepVent,
- but no problem with Tag alone (pg 88).

used: DeepVent buffer - 2 mM Mg  
200 μM dNTP  
0.15 μM primer  
100 pg template (10 pg/μl)

| Tag: |     |      | Tag + DeepVent |     |           | DeepVents |    |
|------|-----|------|----------------|-----|-----------|-----------|----|
|      |     | Unit | #              |     |           | #         |    |
| 1    | 2   | 0    | 31             | 32  | 10 + .001 | 15        | 16 |
| 3    | 4   | .5   | 33             | 34  | .005      | 17        | 18 |
| 5    | 6   | 1    | 35             | 36  | .01       | 19        | 20 |
| 7    | 8   | 1.5  | 37             | 38  | .05       | 21        | 22 |
| 9    | 10  | 2    | 39             | 40  | .1        | 23        | 24 |
| 11   | 12  | 2.5  | 41             | 42  | .2        | 25        | 26 |
| 13   | 14  | 5    | 43             | 44  | .5        | 27        | 28 |
| 15   |     |      | 45             | 46  | 1         | 45        | 46 |
|      | 15x |      |                | 17x |           | 47        | 48 |

10x buffer 75  
dNTP 15

Template 150  
primer 1 3.75  
2 3.75

50 μl / Rx

Cocktail → 50 μl / Rx

enzyme diff. amount  
D.V. + 10 Tag in all

20x

50 μl

diff. am  
2  
deep vent

added enzyme  
separately in 1 μl

To Page

Witnessed & Understood by me,

Date

12/1/94

Invented by

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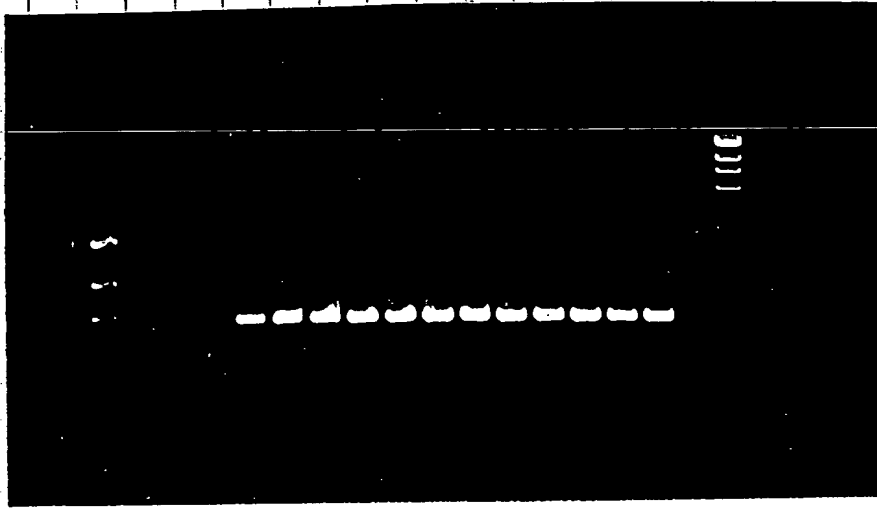
K. Sturman

Date

11/30/94

ag No. \_\_\_\_\_

Tag

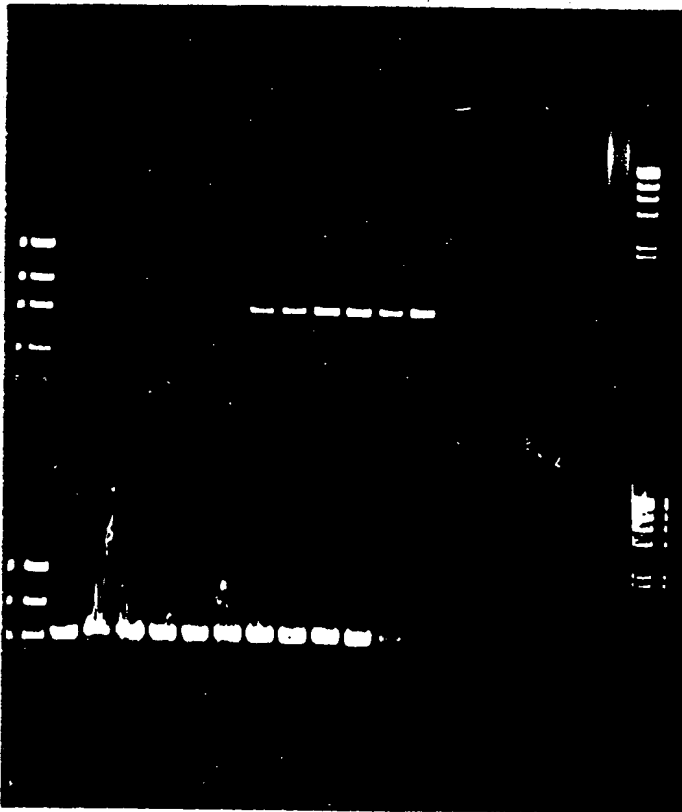


Result:

- 100 pg + 25 cycles seem to be enough
- Deepvent alone with regular primer worked again
- Deepvent at lower concentration works better. with 0.05 V good product yield was seen.

.001 .005 .01 .05 .1 .2 .5 1 2

*MM*



- So may be with earlier run, if the enzyme con. is lower, it might have worked with Deepvent. Try?

10 Tag + .001 .005 .01 .05 .1 .2 .5 1 V Deepvent

To Page No. \_\_\_\_\_

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Dat

12/19/94

Invented by

Date

12/1/94

Recorded by

K. S. Araman

Project N \_\_\_\_\_  
 Book N \_\_\_\_\_

representing with Tne

Page No. \_\_\_\_\_

32P23m (p75)

5.3

✓

PUC18 RF

10.5

✓

10x Tag buffer

~~3.5~~ 4.5

✓

H<sub>2</sub>O

14.5

✓

Tne 2.4  $\mu$ l

1

✓

dilute in Tag storage buff

3.6  $\mu$ l

✓

8.5 8.5 8.5 8.5

ddA

2

C

2

G

2

T

2

To Page No. \_\_\_\_\_

ed & Understood by m ,

naa a Polarp

Date

11/29/94

Invented by

Recorded by

Date

11-2-94

Project No. \_\_\_\_\_

Book N . \_\_\_\_\_

TITLE \_\_\_\_\_

From Pag No. \_\_\_\_\_

cycle reg.  
the pool

ACCTACCT

Vent buffer Cheng Buffer

HF1

min

0 2 5 10 20 60

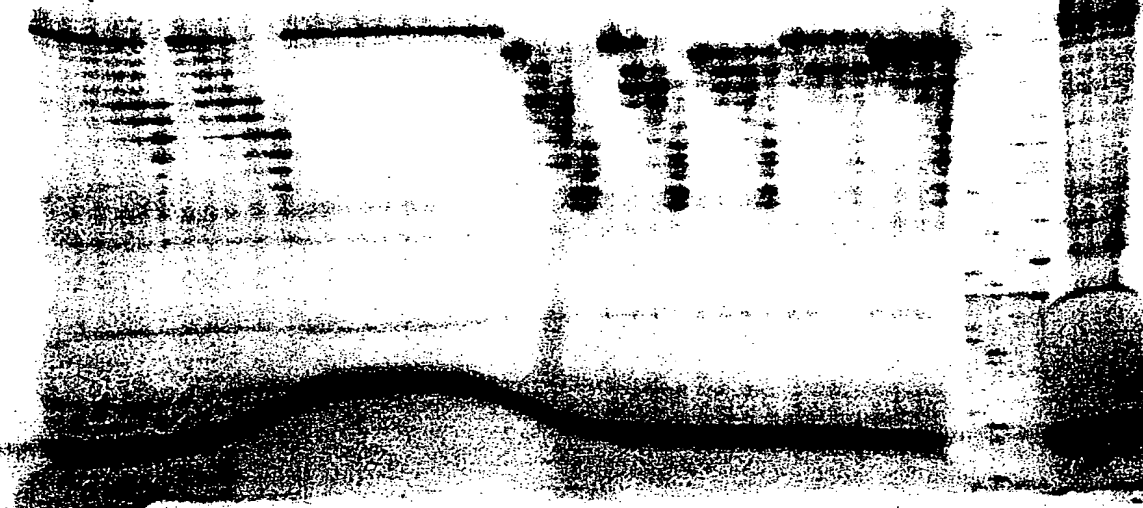
2 5 10 20 60

2 5 10 20 60

2 5 10 20 60

-- -r S- SR -S

10 3 10 60 10 3 10 60 10 3 10 60 10 3 10 60



2/11/94 3 211-10,000

stage

To Pag

Witnessed & Understood by me,

Date

11/29/94

Inv nted by

R c rded by

Date

11-2-94

Tue. 8-5 8x minus.

3-5 210  
Tue 3/30/95

Page No. \_\_\_\_\_

Track BFR (Tel) - Suspended 11.5 grams in 30mls in BFR

50mm Tris - 7.5 - 100ml  
0.5mm EDTA - 2ml  
0.2mm PMSF - 4ml  
8.5g glycerol - 100ml  
5mm Bml - 6.99ml  
10mm kcl - 6.7ml

Sandata w/ med. TIP - output - 8-  
3x 1min -  
1:200 w/ dltw <sup>RD</sup> 595  
Crude = 0.74  
#3 = 0.17

Heat kill 85°C for 10 min  
Cool - 0 Make 50mm kcl (phosphate conc.)  
© ADD 0.4% PEST.

ach vol = 40mls = 660ml kcl  
1.6 ml 10% PEST.

→ spin in 55-57 15min - 20G's -  
- Decant Sup 33 mls  
60% Nky 50% cut = 390g/l = 12.5

→ 1.75ml/wl Resin  
Equilibrate 8ml B50 Hydrate Calcium w/ - 8ml = 3.44 x 0.25 =  
8ml = 0.785 = 10.2 cm


2- 2.5ml Tris - 7.4  
8.5g glycerol -  
0.5mm EDTA  
10mm kcl  
5mm Bml  
0.5mm PMSF.

Resuspend pellet 1:1 w/ A -  
- Dialyze 10hr - in A - vs. 20 vol's -  
- 12.5T gradient = 96 mls -  
1.6 ml/fraction x 60 fractions

Same (+) 1.5ml kcl.

gradient = 1.75 ml/wl -  
55 min -

To Page N \_\_\_\_\_

|   |                 |  |                 |
|---|-----------------|--|-----------------|
| sed & Understood by me,<br><br>May Long | Date<br>5/31/95 | Invented by<br> | Date<br>5-22-95 |
|   | R corded by     |  |                 |

Proj ct No. \_\_\_\_\_

Bo k No. \_\_\_\_\_

13

ag N \_\_\_\_\_

2/21/95 TUE

DIGEST T.nea/pSPORT with SstI & SphI

DIGEST M13 mp18 & M13 mp19 w/ SstI & SphI

M13 mp18 RF (0.44 µg/µl) } cut 500.0 ng  
M13 mp19 RF (350.0 µg/ml) }

$$\rightarrow 1000 \text{ ng/} \mu\text{g} \times 0.44 \mu\text{g} = 440 \text{ ng}$$

$$\frac{500 \text{ ng}}{440 \text{ ng}} = 1.1 \mu\text{l}$$

$$\rightarrow 1000 \times 0.350 \mu\text{g/} \mu\text{l} = 350 \text{ ng}$$

$$\frac{500 \text{ ng}}{350} = 1.4 \mu\text{l}$$

mp18

mp19

|                                 |                            |
|---------------------------------|----------------------------|
| H <sub>2</sub> O - 35.0 µl      | H <sub>2</sub> O - 35.0 µl |
| 10x buffer - 2.0 µl ← REact 2 → | 10x buffer - 2.0 µl        |
| 500ng DNA - 1.1 µl              | DNA - 1.4 µl               |
| 1µl SstI - 2.0 µl               | SstI - 2.0 µl              |
| 40.0 µl                         | 40.0 µl                    |

T.nea/pSPORT

H<sub>2</sub>O - 81.0 µl  
10x buffer - 10.0 µl  
ng/µl DNA - 4.0 µl  
SstI - 5.0 µl  
100.0 µl

- Incubated all 3 tubes @ 37°C for 1/2 hour
- Made 0.8% agarose gel  
250.0 ml TE buffer  
2.0 g Agarose
- boiled for 4.0 min.
- added 12.0 µl E. Bromide
- poured the gel.

To Page No. \_\_\_\_\_

sed & Understood by me,

Date

Invented by

Date

4/12/95

Recorded by

4/12/95

SD okup

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_ TITLE \_\_\_\_\_

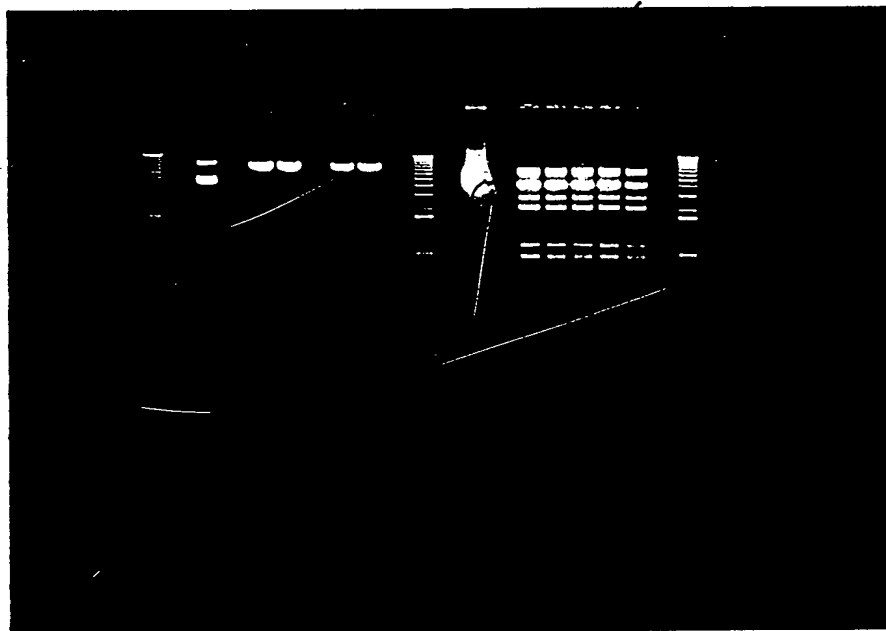
From Page No. \_\_\_\_\_

added: 2.0  $\mu$ l of 1M KCl      2  $\mu$ g = 2000 ng  
to 40.0  $\mu$ g (mp 18 & 19)

5.0  $\mu$ l of 1M KCl  
to 100.0  $\mu$ g (pSPORT)

added ~~Sph~~ Sph I - 2.0  $\mu$ l mp 18  
2.0  $\mu$ l mp 19  
5.0  $\mu$ l pSPORT

- Incubated @ 37°C for 1/2 hour
- put the tubes in the fridge till
- ran samples on the gel ~~also~~ on 2/22/95



arp 2/22/95 ①

M13mp18 & mp19 RF D  
are ds, supercoiled forms  
the DNAs of phages M13  
& 19. Using this vector  
foreign DNA can be  
inserted into the mul  
cloning site in an  
oriented fashion.

T Pag N

With ssed &amp; Understood by m ,

*[Signature]*

Date

4/12/95

Invented by

R c rd d by

*[Signature]*

Date

4/12/95

116

12/1/94

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

From Page No. \_\_\_\_\_

Purpose: - To try new primers again with pM19  
- to get rid of mispriming optimization of Mg.

used: KlenTag buffer w/o Mg added Mg later, different concs 5 µl.

|                 |                    |    |           |      |    |      |
|-----------------|--------------------|----|-----------|------|----|------|
| 1 Unit tag      |                    |    |           |      |    |      |
| 1 µM primer     | included           | mM | 1         | 1.5  | 2  | 2.5  |
| 200 µg template | primer 1 alone     |    | 5         | 7.5  | 10 | 12.5 |
| 200 µM dNTP     | 2 "                |    | 45        | 42.5 | 40 | 37.5 |
|                 | w/o primer         |    | 50        |      |    |      |
|                 | w/o Mg as controls |    | 1         |      |    |      |
|                 |                    |    | 5 µl / rx |      |    |      |

Cycling: 30 ( 94° 3', 94° 30", 56° 30", 72° 3' )  
72° 30"  
4°

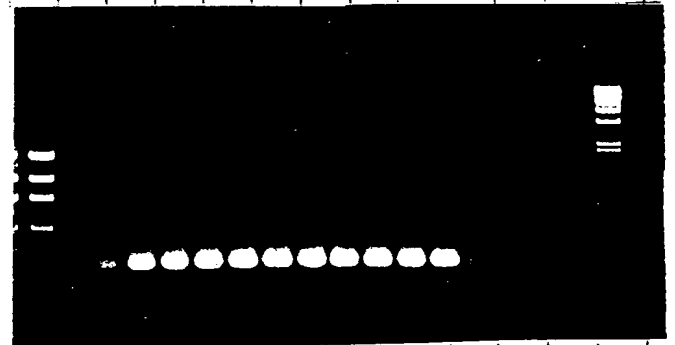
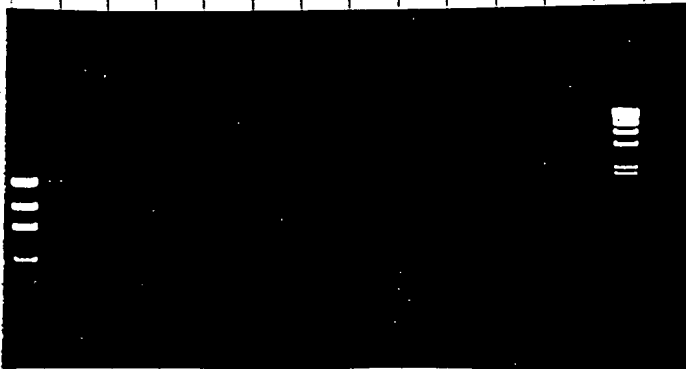
|                  |       |
|------------------|-------|
| 10 x buffer      | 60    |
| dNTP             | 120   |
| Template         | 2.4   |
| enzyme           | 2.4   |
| primer 1         | 6.0   |
| 2                | 6.0   |
| H <sub>2</sub> O | 451.2 |
|                  | ↓     |
| 45 µl + 5 µl     | Mg    |

- Did the same with new dU primers.

2728  
2729

10.7 + 10.6 primers 1 & 2  
1441.9 H<sub>2</sub>O

w/o primers assembled separately



Witnessed & Understood by me,

Date

12/1/94

Invented by

Recorded by

Date

12/2/94

To Page



Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

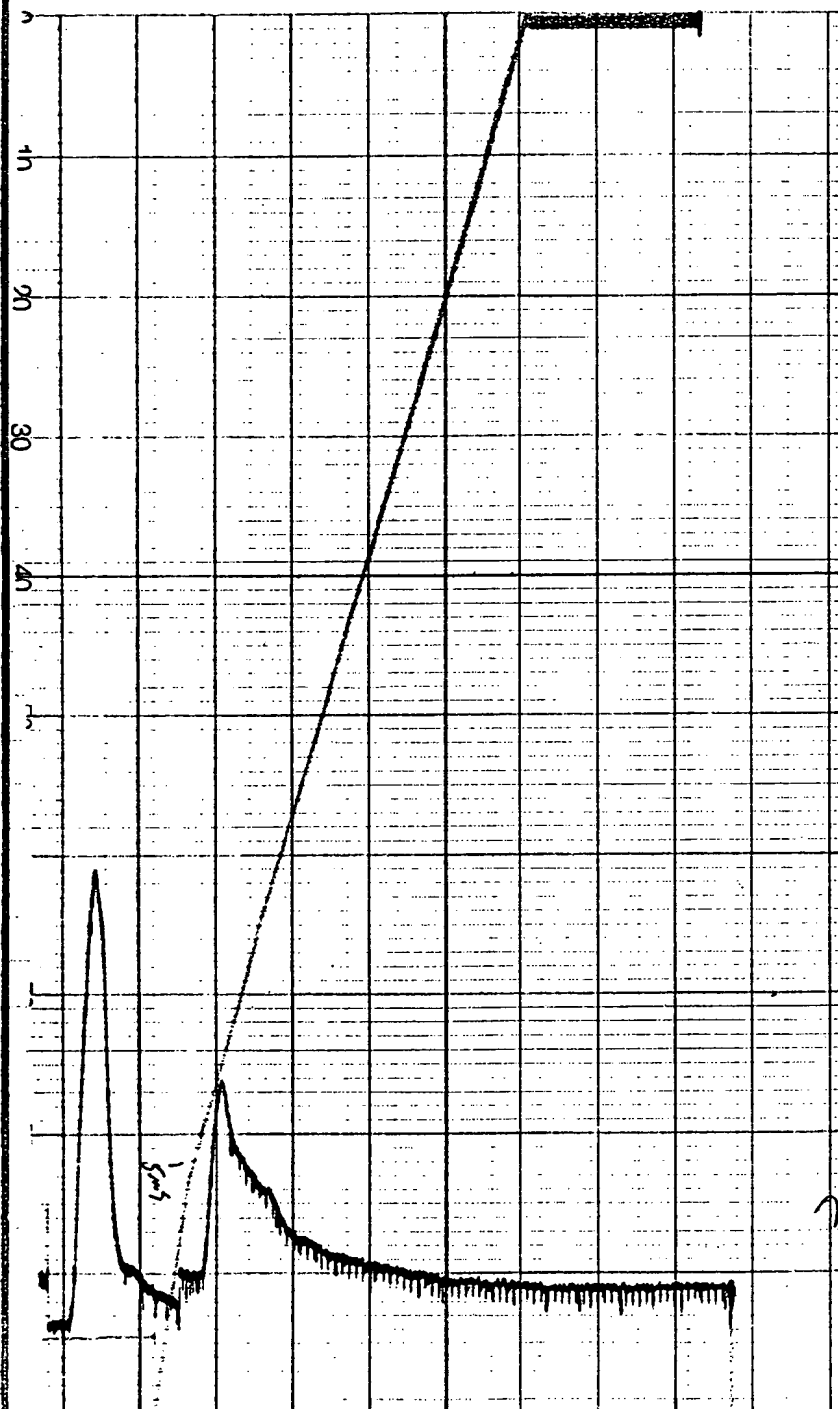
TITLE

*TR50 HEP 650 ml -*  
~~Repeat of Ind Assay - Enzyme~~  
*Curve*

130

From Page No. \_\_\_\_\_

*8ml elution -*  
*Gross column -*



*Curve*  
*Post H.D.*  
*Am 503-549*

*20 AD**WFT**WFT**4**6**8**10**12**14**16**18**20**22**24**26**28**30**40**60**105**110**115*

|    |        |
|----|--------|
| 1  | 892.00 |
| 2  | 440.00 |
| 3  | 198.00 |
| 4  | 80.00  |
| 5  | 74.00  |
| 6  | 72.00  |
| 7  | 70.00  |
| 8  | 64.00  |
| 9  | 92.00  |
| 10 | 82.00  |
| 11 | 62.00  |
| 12 | 86.00  |
| 13 | 74.00  |
| 14 | 58.00  |
| 15 | 102.00 |
| 16 | 58.00  |
| 17 | 58.00  |
| 18 | 96.00  |
| 19 | 48.00  |
| 20 | 96.00  |
| 21 | 80.00  |
| 22 | 64.00  |
| 23 | 66.00  |
| 24 | 100.00 |
| 25 | 64.00  |
| 26 | 102.00 |

*MY*  
*5/31/95*

*MY*  
*5/31/95*

Pharmacia LKB Biotechnology

Code No. 18-1001-44

To Page

Witnessed &amp; Understood by me,

*Amey Lomzo*

Dat

*5/31/95*

Invented by

*Rec rd d by*

Date

*5-31-95*

age No. \_\_\_\_\_

- Sowdick 2x - dilute #3 -  
 - Fyngl Thaw 2x - dry ice -

Eggs - A =

25 mm Tiz - 7.4

81 g/gal

0.1 mm EDTA

20 mm kel

5 mm Base

- Heat kill 85°C - 10 min -

- Assay pre + post H<sub>2</sub>O treatment

1 104.00

2 150.00

3 104.00

4 128.00

5 9012.00

6 146.00

7 114568.00

= Same (F) 1.2 m/kel

To Page No. \_\_\_\_\_

Read &amp; Understood by me,

Date

Invented by A

Date

May Jongo

5/31/95

Recorded by

5-2595

80

Project No. \_\_\_\_\_  
Book No. \_\_\_\_\_

TITLE 23mer degradation: V, DV, Inc  
buffers: Cheng vs Vent vs. KlenTag

From Page No. \_\_\_\_\_

① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩ ⑪

|                           |      |    |      |      |      |    |   |   |   |    |   |
|---------------------------|------|----|------|------|------|----|---|---|---|----|---|
| Cheng buffer 5X           | 20   | →  |      |      |      |    |   |   |   |    |   |
| 10x KlenTag buffer *      |      |    | 10   | →    |      |    |   |   |   |    |   |
| Vent buffer               |      |    |      |      |      | 10 | → |   |   |    |   |
| Tog storage buffer        |      |    | 2    | 2    | -    |    |   |   |   |    |   |
| Mg OAc 12 mM              | 9.5  | →  |      |      |      |    |   |   |   |    |   |
| Mg SO <sub>4</sub> 100 mM |      |    | 1.2  | μl   | →    |    |   |   |   |    |   |
| glycerol 50%              |      |    |      |      |      |    |   |   |   | 16 | → |
| DMSO 100%                 |      |    |      |      |      |    |   |   |   |    |   |
| 32P 23mer **              | 3    | μl | →    |      |      |    |   |   |   |    |   |
| Vent pol 0.05 μl          | 2    |    |      | 2    |      | 2  |   |   | 2 |    |   |
| Deep Vent 0.05 μl         |      | 2  |      |      | 2    |    |   | 2 |   |    | 2 |
| Tne 0.5 μl                |      |    | 2    |      |      | 2  |   |   | 2 |    |   |
| H <sub>2</sub> O          | 6.55 | →  | 81.8 | 81.8 | 83.8 | 85 | → |   |   | 69 | → |

Preheat to 70°C, start by addition of DNA pol  
remove 10 μl to 5 μl cycle reg stop mix at 10, 20, 30 min  
well #1 is 23mer uncut

To Page

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Deena Pokany

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11/29/94

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(14) (15)

✓  
✓  
✓

✓ ← (note KlenTag requires reagents on Tag storage buffer for glycerol and  
Tweens/NP40 - for TAE it is diluted in Tag storage buffer  
so no supplement is needed for Vent  
and Deep Vent dilution  
is in storage buffer  
(with Triton and  
50% glycerol)

✓ (1.2 mM Mg OAc Cf)

✓ (1.2 mM MgSO<sub>4</sub> Cf)

✓ Cf = 8% glycerol

✓ Cf = 2% DMSO

✓

} dilute in Vent/Deep Vent storage/dilution buffer (its  
2.1% Triton)  
(dilute in Tag storage buffer) 0.5% Cf = 0.002%  
Triton  
will include  
2 µl Tag storage  
buffer next time  
(similar to TFL  
storage buffer with  
0.5% Tween/NP40)

✓

2 µl, 0.66 pmol  
3 µl, 0.66 pmol  
12.0 µl, 0.66 pmol  
13.5 µl (8.91 pmol)  
16.8 µl (2.5 pmol)  
5.5 µl  
0.36 pmol primer

\*\* for 72p23 mix 0.66 pmol/1 13.5 µl  
plus 16.8 µl cold 5'3' 23 mix plus  
24.7 µl H<sub>2</sub>O. 0.5% V<sub>f</sub> = 55 µl and specific  
activity is reduced 2x

\* 10x KlenTag is 500 mM Tris HCl pH 9.0  
160 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and no MgSO<sub>4</sub>

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maria Polanco

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11-4-94

1g N \_\_\_\_\_

2/22/95

1. grow cells overnight (O/N) 10.0 mL

= 9.0 mL (1.0 mL in ea. nine tubes). Each tubes labelled AH10B

- Quick freeze all nine tubes in a powdered dry ice.

P12C1T.nea

2/22/95 BJS

LB + AP100

GENE CLEAN

2) Did electrophoresis for yesterday's DNA (2/21/95)

M13 mp 18 and M13 mp 19 and pSPORT

b) Took the picture of the gel

c) cut off mp 18 fragment, mp 19 fragment & pSPORT fragment from the gel & transformed the gel w/ <sup>pa</sup> DNA into the separate eppendorf tubes.d) added 700.0  $\mu$ L NaI to each <sup>2</sup> tubes. Vortexed mp 18 & mp 19 tubes.

e) Incubated both tubes @ 55°C to melt agarose. mixed after incubation

f) added 5.0  $\mu$ L glass milk to both tubes.

g) Incubated both tubes on ice for 5 min.

h) cfg. both tubes (quick spin)

i) discarded supernate

j) added 500.0  $\mu$ L New Wash bufferk) discarded supernate & again added 500.0  $\mu$ L New Wash buffer. washed both tubes 3 times.l) added 10.0  $\mu$ L dH<sub>2</sub>O to the tubes. mixed well by vortexing. 55°C for 2-5 min

m) set up Ligation

Ligation

H<sub>2</sub>O = 12.0  $\mu$ LH<sub>2</sub>O = 12.0  $\mu$ Lligase) 5X Buffer = 4.0  $\mu$ L5X buffer = 4.0  $\mu$ Lmp 18 DNA = 2.0  $\mu$ Lmp 19 DNA = 2.0  $\mu$ L(1  $\mu$ L) Ligase = 2.0  $\mu$ LLigase = 2.0  $\mu$ LTV = 20.0  $\mu$ LTV = 20.0  $\mu$ L

n) Incubated both tubes overnight @ room temperature (cond)

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(con'd)

T.nea/Ptnc E. pttc

- 1.0 mL of ea.
- cfg.
- discarded supernate
- added 100.0  $\mu$ l SI mixed well
- Incubated on ice for few min.
- added 200.0  $\mu$ l S2 lysis
- Incubated on ice for few min.
- added 150.0  $\mu$ l S3 w/ RNAase A
- cfg. for 7.0 min. @ 4°C

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Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

118

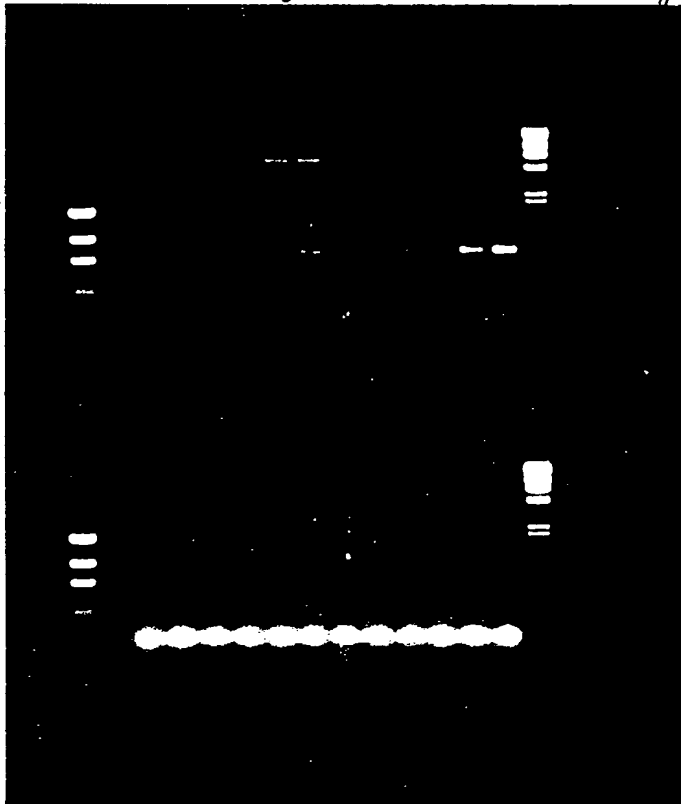
12/1/94

From Page No. \_\_\_\_\_

Purpose: Since 1U of Tag - titration with Mg didn't work, increase the amount of enzyme to 2U.

- Both new + old dV primers were tried.
- Expt was done under same condition as I.

old dV 0 1.5 2.5 2.5 2.5 3.0 mM Mg



new dV

Result:

- mispriming persists
- 2U better than 1U
- 2U / 1.5 mM Mg ✓
- again new dV for didn't work - can give up.

\* Try again with new dV primers & increasing amount Tag & Mg.

To Page 1

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Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

From Page No. \_\_\_\_\_

- used Max efficiency DH5 $\alpha$  ~~8~~ RK3 101 to transform
- control puc 5  $\mu$ l = 50 pg } + 100  $\mu$ l of Competent
- 5  $\mu$ l of each UDG Rx
- add 900  $\mu$ l of <sup>50c</sup>competent cells and let  
to express for 1 hr.
- plated control - diluted 1:10, 20, 50, 100  $\mu$ l  
Test - unv, 20, 50 & 100  $\mu$ l.
- 100  $\mu$ l from each plated x 2
- Agouti counted them all.
- App - depletion resulting in  
- lab of satellite colonies - to  
to score.
- According to Agouti in Mr treated the counts were ~ 50 -
- Rest not much difference between balanced + input into  
reaction, ~~was~~ still more whites than (normal) and  
this of mine.
- ~ 10 fold increase in the present set of plates  
= ~ 10% whites.
- plated a few more from each reaction for better  
accurate count. These plates were treated with 50 pg  
of fresh Ampicillin.
- prepared in total of 100 myl ml.

12/6/94

To Page 1

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Date 12/6/94

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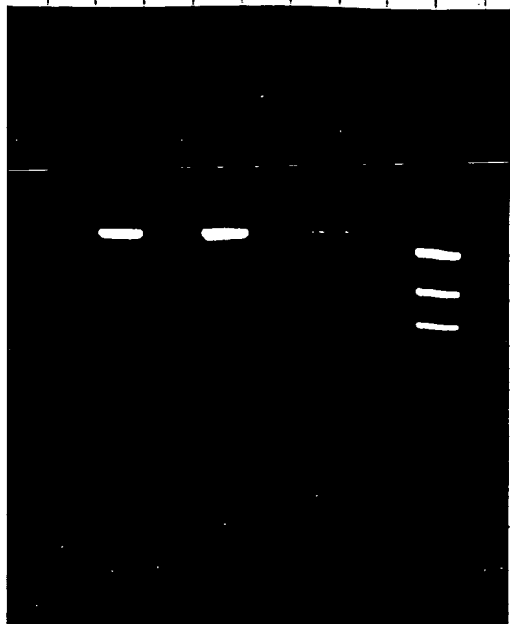
Date

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b 2 b 10



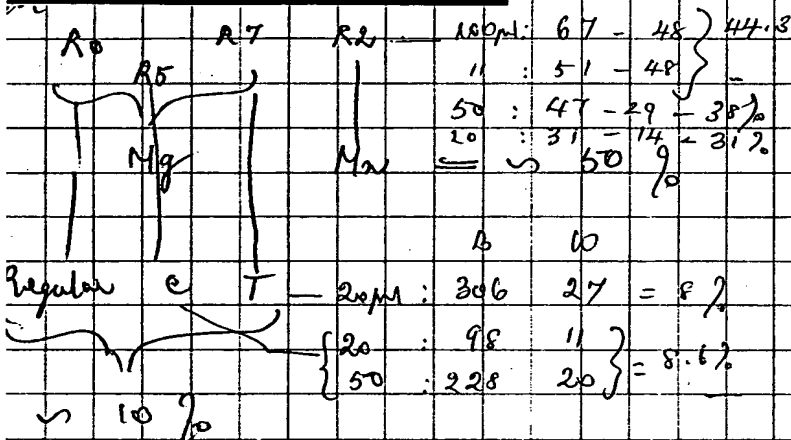
50 ng  
30  
20  
10  
5  
2.5

- agent did another reaction with Tag, different cycle # and 2 dif. con of target template; 5 pg and 50 pg with du primers & pvc.

- % cycles: 10, 20, 35 & 40

- with 10 cycles no product in both con of target

with 20 cycles, 5 pg target barely visible product 50 pg gave a faint amount which was quite visible.



Regular c T - 20 pm: 306 27 = 8%  
20 : 98 11 } = 8.6%  
50 : 228 20 }

both 5 & 50 pg quite a good amount of product yield with 35 & 40 cycles. With 50 pg more than 5 pg & 40 cycles more than 35 cycles as expected.

Did transformation of all the products more amp spread out 500 µl plate.

All with Tag.

Result: colony % too low even at 35 & 40 cycles. But high % of colonies unexpectedly high even at lower # of cycles??

np: no problem - even in BPP plates satellite colonies with Ax & not with control something to do with test reactions + transformation  
colony yield did not correspond w % of colonies obtained?  
correlation between % of cycles and % of errors??

To Page N \_\_\_\_\_

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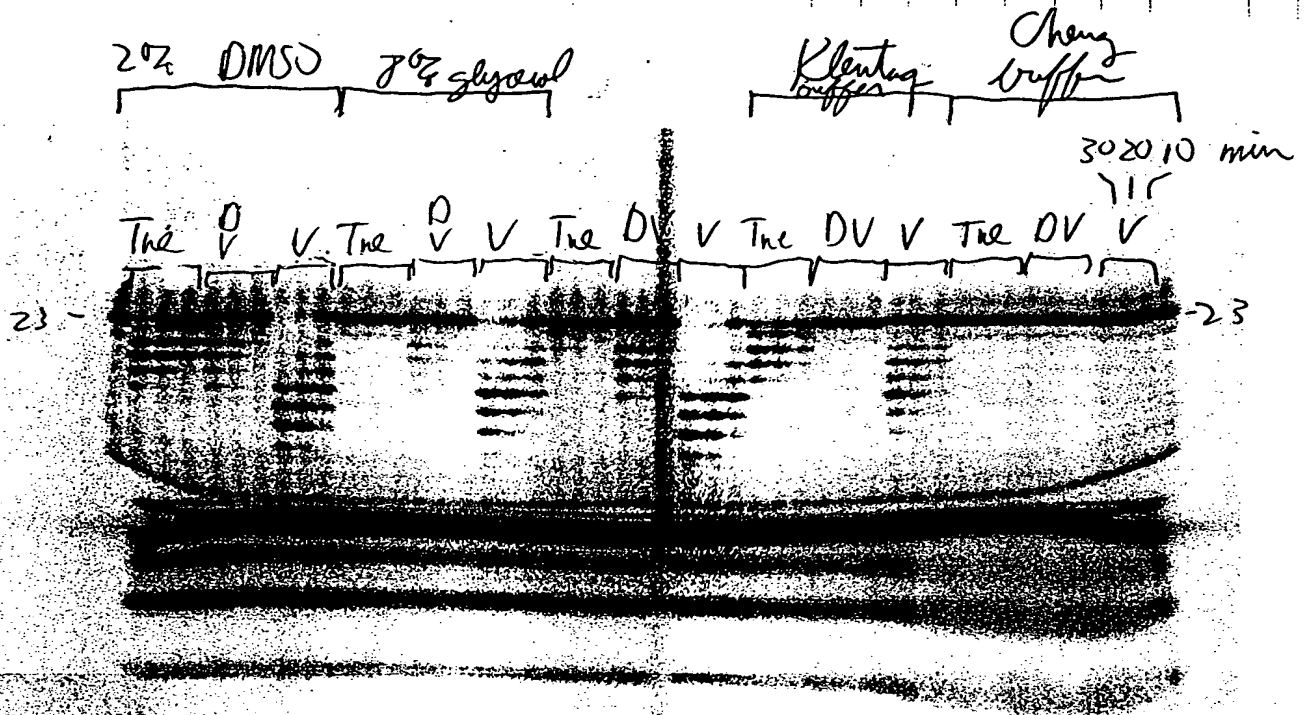
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Dr. Silasamun

From Page No. \_\_\_\_\_



Result.

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11/5-94

From Page No. \_\_\_\_\_

| 20% DMSO  |       | 70% glycerol  | Klentz buffer | Cheng buffer |
|---|-------|---------------|---------------|--------------|
| Tris pH 8.7                                     | 20 mM |               |               |              |
| Tris-HCl pH 7.1                                 |       |               |               |              |
| K <sub>2</sub> SO <sub>4</sub> pH 8.7           | 85    |               |               | 20 mM        |
| KCl   |       |               |               |              |
| (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> |       |               |               | 10           |
| Mg(OAc) <sub>2</sub>                            | 1.2   | 1.6           |               | 10           |
| MgSO <sub>4</sub>                               |       | 1.2           |               | 2            |
| DMSO  | 2%    |               |               |              |
| Tricine   |       |               |               | 0.1%         |
| Tris-20/MPD                                     |       | 0.1% from top |               |              |
| glycerol  | 8%    | 1             |               | 1            |
| some stuff                                      | 1.05  | 82            |               | 50           |
| Vault   | 1     | +             |               | ++           |
| Deep vault                                      | 1     | 1             |               | +            |
| Tris  | 1     | +             |               | 1            |

Re

(+ for DMSO)

To Page N

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Deena Polay

Date

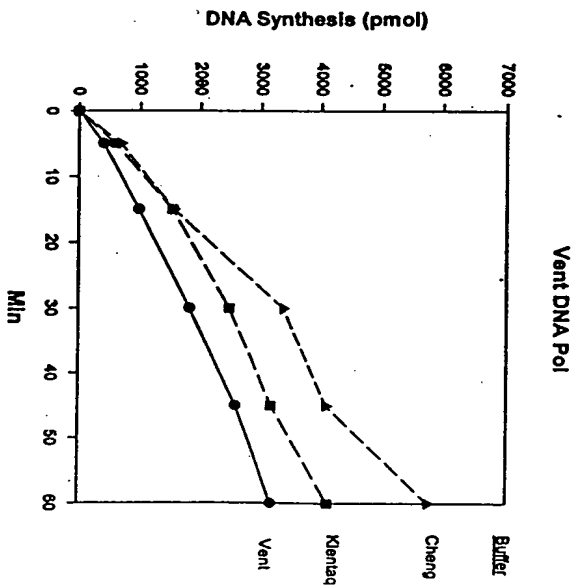
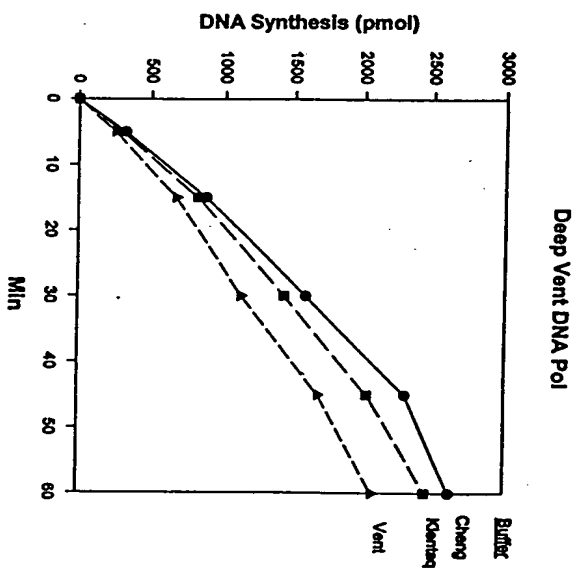
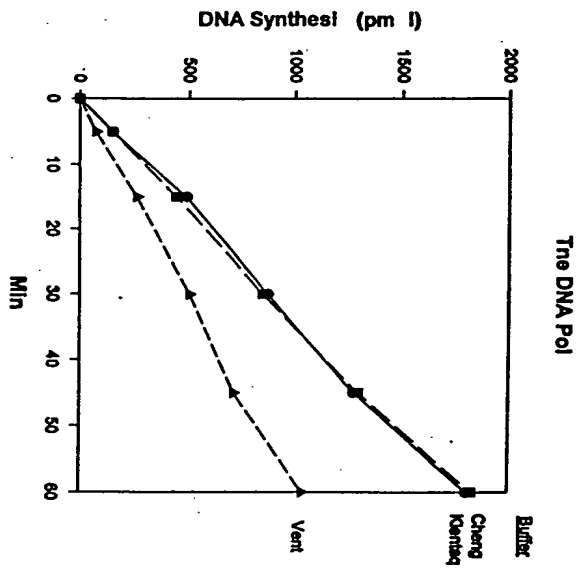
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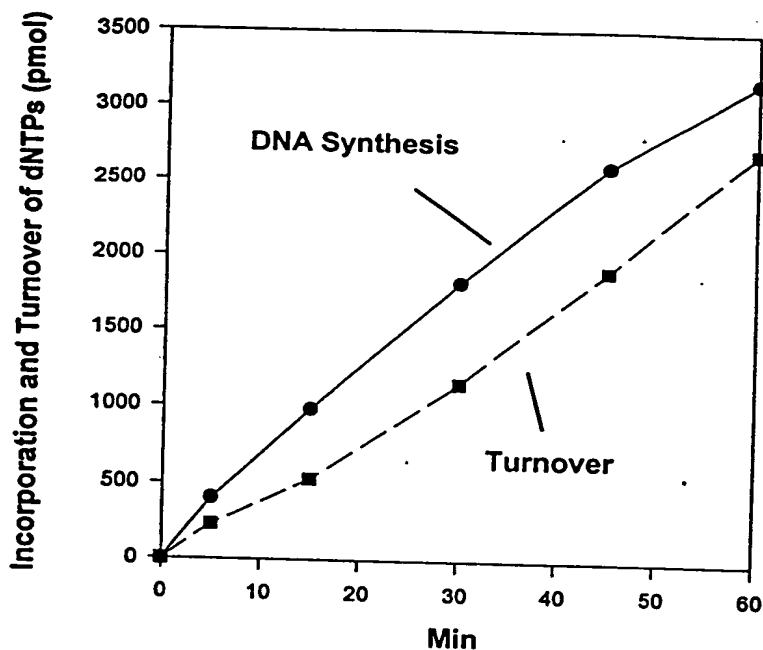
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In each case, DNA synthesis is lower in  
 Primer degradation was highest in Vent

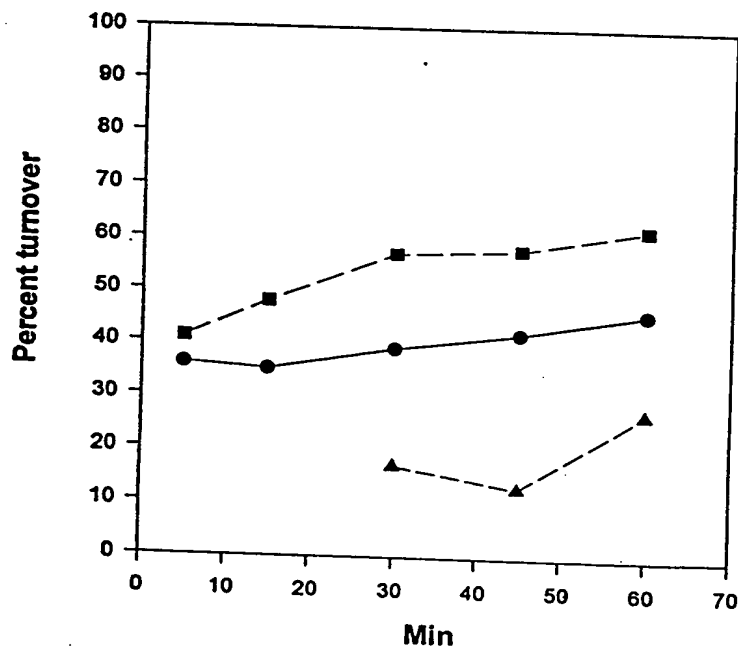
got Turnover  
 by DNA synthesis  
 1, below

Vent DNA Pol in Vent Buffer



DNA synthesis  
and turnover  
to dNMP

Activity in Vent Buffer



DNA Pol

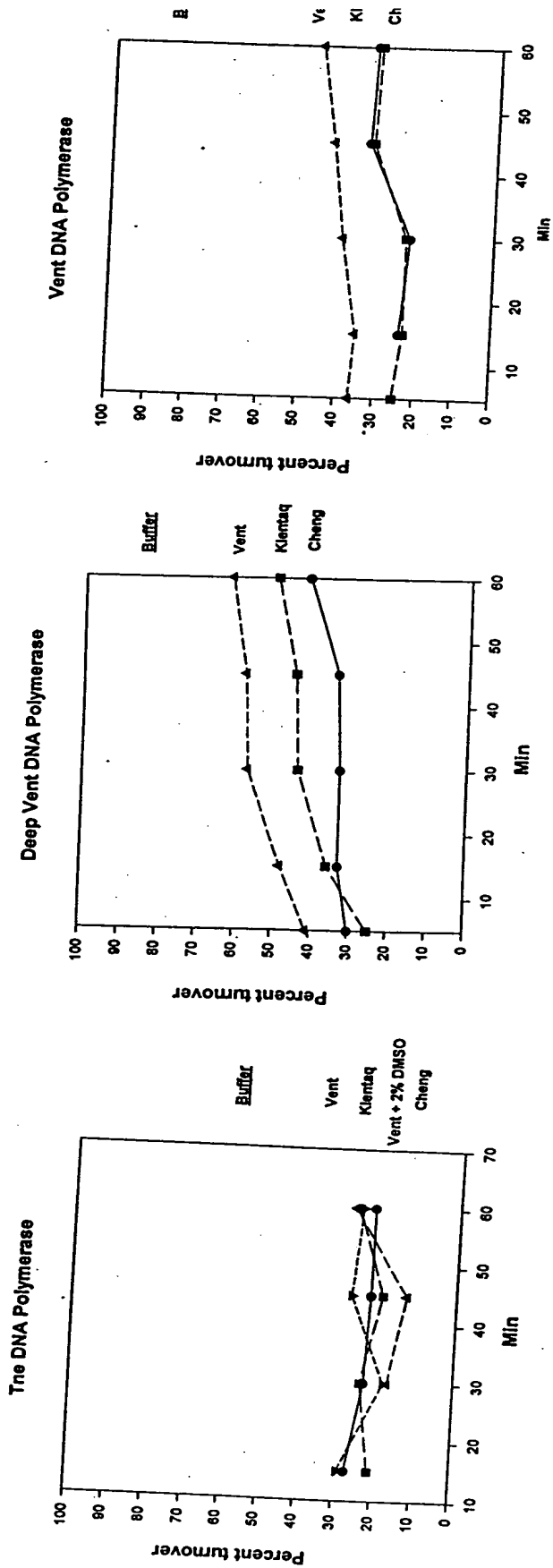
Deep Vent

Vent

Tne

$$\frac{\text{Percent turnover}}{\text{incorporation} + \text{turnover}}$$

Deep Vent has  
higher turnover  
than Vent as  
expected. Tne  
is ~2x lower  
than Vent and  
Deep Vent



effect of buffer on turnover is not large compared to effect on primer degradation

ssed & Und rstood by m ,  
erica Polcup

Date  
11/29/94

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11-5-94

Project No. \_\_\_\_\_  
Book No. \_\_\_\_\_

TITLE New 3'-5' exo nuclease Mutant of T<sub>4</sub>

From Page No. \_\_\_\_\_

4/13 -

Purpose: ~~See~~ Previous clone (P. 129) of a 3'-5' exonuclease mutant of *Thermotoga neopolitana* (Tne) proved not to ~~produce~~ over express a heat sensitive or no polymerase activity. ~~More Gre Dels~~ ~~producers~~ made a new clone. The purpose of his experiment is to screen pre + post heat kill for a polymerase activity. If activity is ~~more~~ thermostable then proceed w/ a PET + (NH<sub>4</sub>)<sub>2</sub> SO<sub>4</sub> ppt.

3 grams of cells - suspend in 1ml of crack buffer -  
Sonicate with ~~mini tip~~ micro tip ~~ultrasonicator~~

crack buffer -

20mM Tris pH 7.5

10mM KCl

1mM EDTA

5mM Bme

5% glycerol

#575 - .824 before crack

A575 - .198 after crack 6x 20sec  
76% crack - minotip C  
setting 4.

Save 400µl → No heat treatment

Aliquot the rest of the cracked material to 2mL eppendorf  
heat kill 10min → temp. @ 80° - 90° C -  
note: temperature rose to > 90 for maybe up to 5min

Spin in microfuge @ 14000g 30 minutes -

Heat treat supernatant < 90° > 85° C - for 5 minutes -  
spin in microfuge @ 14000 x g 10 minutes -

To Page 1

With ss d & Understood by m ,

May 20/95

Date

6/20/95

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6/13/95

ag N. \_\_\_\_\_

06/13/95

say in thermostable polymerase activity -

mix - TAD premix - premade by A.G. -

add 1.1  $\mu$ l / 500  $\mu$ l premix  
of 2320 dCTPDilution  $5/495 - \frac{10}{90} - \frac{1}{1000}$   
 $290 - \frac{1}{3000}$  $1/1000$  1  $\mu$ l  
2  $\mu$ l heat treated $1/3000$  1  
2 $1/1000$  1  
2 before heat treatment $1/3000$  1  
2 mistake made - put 78  $\mu$ l of premix should have only used 48 !!incubate 10' @ 74°C in a heated water block - quench rxn w/ 10  $\mu$ l of 5M EDTA - spot 30  $\mu$ l on 6H/C 11.2i

wash filters

1x 10% TCA 5'

3x 5% TCA 3'

2x 5 to H

dry + count in econofluor LSF

3M CPM1

1  $1/1000$  2994.00

2 2384.00

3  $1/2000$  622.00

4 888.00

5  $1/1000$  3612.00

6 5296.00

7 1234.00

8  $1/1000$  1662.00

9 964.00

10 90094.00

11 89736.00

12 89120.00

13 40.00

H Kill

No H Kill

S.A

First approx. - looks as though not as much activity after heat &amp; kill - need to do less dilutions to in order to ascertain what exactly is going on.

seeing most polymerase act. in No Heat Kill?

Repeat w/  $1/500$   
 $1/200$  dil's -

mz

0/20/95

T Page No. \_\_\_\_\_

is d &amp; Understood by me,

Date

Invented by

Date

Recorded by

Nay Tonyo

0/24/95

Elizabeth Flynn

06/13/95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

20

From Page No. \_\_\_\_\_

2/28/95 TUE

I set up digest DNA ppt.

① M13mp 18, ② mp 19 and ③ T. nea / pSPORT

1. - To ea 3 added 100.0  $\mu$ l TE  
" " 10.00  $\mu$ l NaAc } to ppt  
" " 300.00  $\mu$ l EtOH } DNA

2. Incubated on dry ice for ~5 min.

3. Cfg. for 10 min. @ room temp. (no ppt) &

4. no ppt., added 2.0  $\mu$ l (carrier molecule) Yeast tRNA. Vortexed

5. incubated on dry ice for ~5 min.

6. Cfg for 10 min. @ room temp. (Supernate saved) Pellet was saved on mp 18 &

7. added 200.0  $\mu$ l 70% EtOH to the pellet

8. Cfg. discarded supernate, air dried by putting tubes on the heat block.

II DIGEST set-up (to map Bam HI site)

- Cut T. nea / pSPORT with Hind III, Bam HI, Xba, NOT I, Sst, EcoR  
Separate

H<sub>2</sub>O - 13.0  $\mu$ l

buffer - 2.0  $\mu$ l

T. nea / pSPORT DNA - 3.0  $\mu$ l.

enzyme - 2.0  $\mu$ l

TV = 20.0  $\mu$ l

Enzymes - Hind III, Xba, Sst had REact 2 1  
buffer.

- Bam HI, NOT I & EcoRI had REact:  
buffer.

Control: H<sub>2</sub>O - 13.0  $\mu$ l

(REact2) buffer - 2.0  $\mu$ l

DNA - 3.0  $\mu$ l

for  
separate  
enzymes.

- Incubated @ 37°C

- ran on the gel on 3/1/95 (Wed)

Picture shown pg 21

To Page N

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ag N \_\_\_\_\_

## I DIGEST set-up

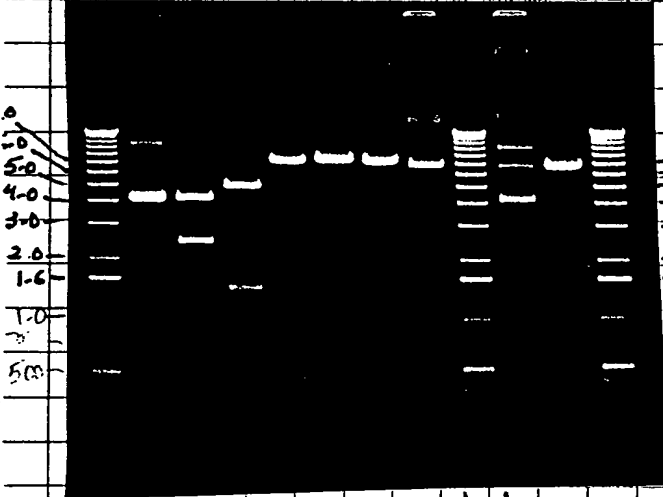
- Cut p<sub>trac</sub> / T<sub>neo</sub> w/ Sst I enzyme

H<sub>2</sub>O - 11.0  $\mu$ l  
 d<sub>2</sub> buffer - 2.0  $\mu$ l.  
 T<sub>neo</sub> DNA - 2.0  $\mu$ l.  
 Sst I - 5.0  $\mu$ l.  
 TV = 20.0  $\mu$ l.

Control: H<sub>2</sub>O - 11.0  $\mu$ l  
 buffer - 2.0  $\mu$ l.  
 DNA - 2.0  $\mu$ l

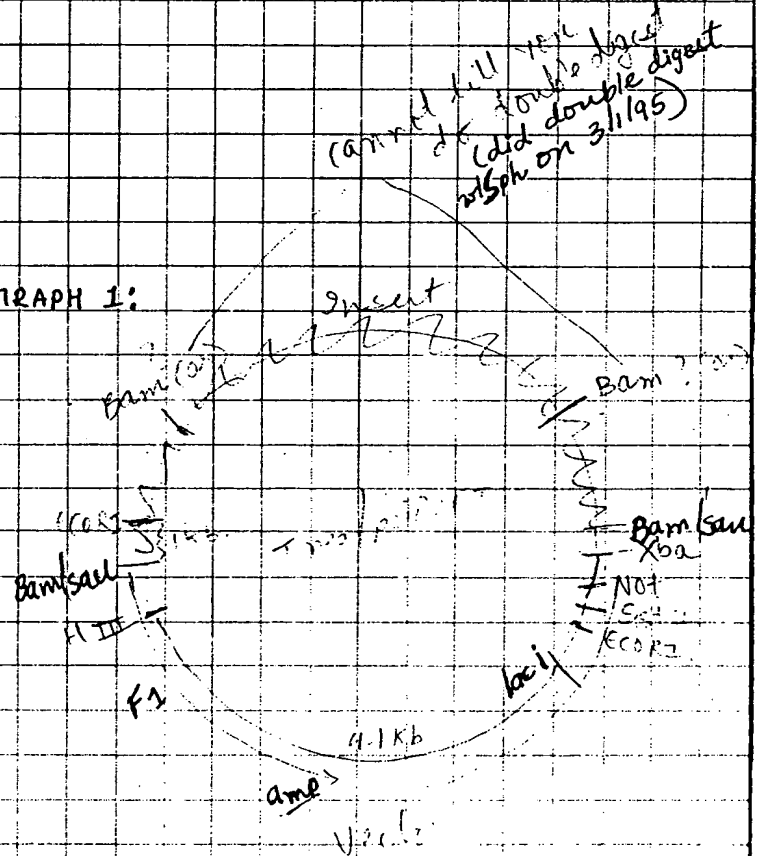
- Incubate @ 37°C

- Lam DNA on a gel on 3/1/95 (wed) picture shown below



from pg. 20

: GRAPH 1:



: GRAPH 2: pg. 23 of this book.

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Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

Turnover for Vent, Deep Vent,  
(follow P. 61, 7)

B4

From Page No. \_\_\_\_\_

H<sub>2</sub>O (A) 399 487 467 489 476  
133 66.7 66.7  
5x Chelex buffer  
10x Klenow  
10x Vent buffer  
Tag storage buffer 6.71 90  
3.7 mg/ml activated DNA  
LATG-TTP 10mM each 3.33  
32P dATP 10mCi/ml 1.21  
Mg(OAc)<sub>2</sub> 50 mM 1.61  
MgSO<sub>4</sub> 100mM 81  
DM50 10.0%  
0.055 ml 0.633 2.633.650 use 180  
(1) (2) (3) (4) (5) (6) (7) (8) (9)  
195 195 195 180 190 150 190 190 19  
4 4 4 4 4 4 4 4 4  
Vent 0.081  
Deep Vent 0.081  
Taq 0.071  
H<sub>2</sub>O 100  
prior to 70°C, start by addition of pol 5 6 6  
remove 151 to 51 0.2 MEDTA → spot 151 on GEL  
and remove 51 to 51 Kill solution (201mol/l DAM  
100 mM EDTA) at 5  
0 5 15 30 45 60 min  
spot 21 on PEI resolve in 1M LiCl  
\* dilutions of pol  
name as P.81

Results: see graph on P.81

T Page N.

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Deena A. Bokor

Date

11/29/94

Invented by

Recorded by

Date

11-9-94

g N

(1)

14.4

✓

✓

✓

66.7 20

✓

→ 27

✓

1  $\mu$ l / 100  $\mu$ l PCR  $\Rightarrow$  Cf = 0.005% Tween 20/NP40

So this makes up for no Tfl here - its present in Joes long PCR Rxn.

→ 1

✓

(Cf = 50  $\mu$ m each)

→ 0.36

✓

(220 x 10<sup>6</sup> total cpm)

✓

(1.2 mM Mg(OAc)<sub>2</sub>)

✓

(1.2 mM Mg SO<sub>4</sub> in Klenow buffer)4  $\mu$ l

Cf = (2% DMSO)

(2 mM Mg SO<sub>4</sub> in 1X Vent buffer)

(10)

19.4

✓

(0.4 units total of each pol)

4

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Date

11-9-94

To Page No. \_\_\_\_\_

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

134

From Page No. \_\_\_\_\_

48  $\mu$ l of premix aliquoted to pre labeled appendants 4/1

|   |       |   |                  |
|---|-------|---|------------------|
| 1 | 1/200 | 1 | } post Heat Kill |
| 2 |       | 2 |                  |
| 3 | 1/500 | 1 |                  |
| 4 |       | 2 |                  |

|   |       |   |                 |
|---|-------|---|-----------------|
| 5 | 1/500 | 1 | } pre Heat Kill |
| 6 |       | 2 |                 |
| 7 | 1/500 | 1 |                 |
| 8 |       | 2 |                 |

incubate @ 74°C in a heated water block - for 10 minutes  
quench w/ 10  $\mu$ l of 5M EDTA - spot 30  $\mu$ l on GPC

Wash 1x 10% TCA 1x Pi 5'  
3x 5% TCA  
2x EtOH

dry + count w/ Econofluor

USER: 2 ID: 32P  
SAMPLE REPEAT:  
H#: 1 AQC: N QCF  
CHANNEL 1-LL:  
DATA CALC: CPM.  
HALF LIFE (DAYS):

| SAM     | CPM1     |
|---------|----------|
| 1/200   | 8460.00  |
| 2       | 21680.00 |
| 1/500   | 8296.00  |
| 4       | 7486.00  |
| 1/200   | 16274.00 |
| 6       | 28614.00 |
| 1/500   | 8412.00  |
| 8       | 17912.00 |
| 9       | 2794.00  |
| 10 S.A. | 91294.00 |

|                  |       |
|------------------|-------|
| } post heat Kill | 11.9  |
|                  | 17.98 |
|                  | 29.7  |
|                  | 28.4  |
| } pre heat Kill  | 30.1  |
|                  | 29.54 |
|                  | 39.7  |
|                  |       |

S.A. 57 cpm/pmol nt

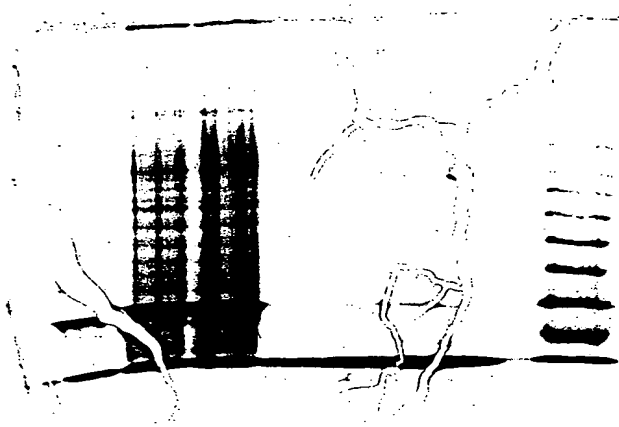
mf  
6/20/95

To Page 1

|  |                 |                                |                  |
|--|-----------------|--------------------------------|------------------|
| Witnessed & Understood by me,<br><br>May Jones | Date<br>6/20/95 | Invented by<br><br>S. A. Jones | Date<br>06/16/95 |
|  |                 | R c rded by                    |                  |

'ag No.\_\_\_\_

127. SDS PAGE - 3'-5' exo minus the



5/10/100

1124 6/20/55

|         |         |         |         |          |          |   |   |   |    |
|---------|---------|---------|---------|----------|----------|---|---|---|----|
| 1       | 2       | 3       | 4       | 5        | 6        | 7 | 8 | 9 | 10 |
| 1 $\mu$ | 3 $\mu$ | 5 $\mu$ | 5 $\mu$ | 10 $\mu$ | 20 $\mu$ |   |   |   | M  |

pre Heat  
Kill

post heat  
kill

u heat - @ 20  $\mu\text{g}/\text{ml}$  - spun down sup loaded on gel  
st heat @ .36  $\mu\text{g}/\text{ml}$

To Page No. \_\_\_\_\_

**ssed & Understood by m ,**

**Date****Invented by**

Date \_\_\_\_\_

May Long

6/20/95

Recorded by

3.4 gm

06/16/95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

#41 - S. Sgm crack

From Page No. \_\_\_\_\_

Purpose: to screen FYI - 1 mutant Tne - one base point mutation phenylalanine to Tyrosine 1 for thermostable polymerase activity.

S. Sgams cells - resuspend in 10 mL of crack buffer - p. 132 ~15

Divide in two 7.5 mL samples in 15 mL conical  
Sonicate w/ microtip @ max output - 5  
6 x 20 sec bursts

Before - A<sub>595</sub> .750 ~ 57% crack  
After - A<sub>595</sub> .320

Sonicate again - 3 x 20 sec bursts

Before A<sub>595</sub> .750 ~ 73% crack  
After A<sub>595</sub> .200

Divide Save 400 mL - pre crack material  
Aliquot remainder of crack into 2 mL eppendorf  
incubate @ 87°C 11 minutes -  
Spin in microfuge 20 minutes @ 14,000

Decant and save supernatant → assay for thermostable polymerase activity -

To 500  $\mu$ L of TAD pre mix add 1.1  $\mu$ L of <sup>p32</sup> dCTP

48  $\mu$ L of pre mix / rxn - 1.2  $\mu$ L of  
diluted samples added incubate @  
74°C in a heated water block -  
for 10'. Quench rxn w/ 10  $\mu$ L of

5M EDTA - spot 30  $\mu$ L of GFC  
Wash 1x w/ 10% TCA 1x w/ 1% Pi @ 5'  
3x w/ 5% TIA @ 3'  
1x w/ EDTA

dry + count -

1 1/200 1  
2 1/200 2  
3 1/400 1  
4 1/400 2  
5 1/200 1  
6 1/200 2  
7 1/400 1  
8 1/400 2

To Page N

Witnessed &amp; Understood by m ,

Date

Investigated by

Date

Recorded by

Mary Longo

6/20/95

E. Flynn

6/16/95

Page N \_\_\_\_\_

USER: 2 ID:32P  
SAMPLE REPEAT:  
H#: 1 ADC:N QCF  
CHANNEL 1-LL:  
DATA CALC: CPM  
HALF LIFE(DAYS)

Note: Background very high!

06/14

Debr note: Did not induce for very long -

| SAM | CPM1     |
|-----|----------|
| 1   | 5722.00  |
| 2   | 7676.00  |
| 3   | 2608.00  |
| 4   | 4686.00  |
| 5   | 10454.00 |
| 6   | 19114.00 |
| 7   | 5850.00  |
| 8   | 11594.00 |
| 9   | 2976.00  |
| 10  | 90102.00 |
| 11  | 88418.00 |

|                |           |           |
|----------------|-----------|-----------|
| Post Heat Kill | 5.8       | 5.4 U/ul  |
|                | 5.0       |           |
| Pie Heat Kill  | 15.9 U/ul | 17.0 U/ul |
|                | 17.1 U/ul |           |
|                | 18.3      |           |

loss of 70%?!

any 6/20/95 Appears to have (very high background) + lost activity after heat kill & maybe mostly host cell activity in pre heat kill?

Brad finds

Slope - .0555 O.D./ug

|                     |    |      |         |            |
|---------------------|----|------|---------|------------|
| Heat Kill Tyl -     | 40 | .474 | * too ↑ | .2 ug/ul   |
|                     | 20 | .333 |         | .289 ug/ul |
| Pre Heat Kill Tyl - | .  | .259 |         | 23.3 ug/ul |
|                     |    | .439 |         | 136 ug/ul  |
| Post Heat Kill      |    | .303 |         |            |
| 3'-5' exo mut       |    |      |         |            |

Run 12x SDS PAGE - see p 138

1-2-3-4  
↓

To Page N \_\_\_\_\_

is d & Understood by me,

Date

Invented by

Date

May Longo

6/20/95

Rec rded by

06/16/95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

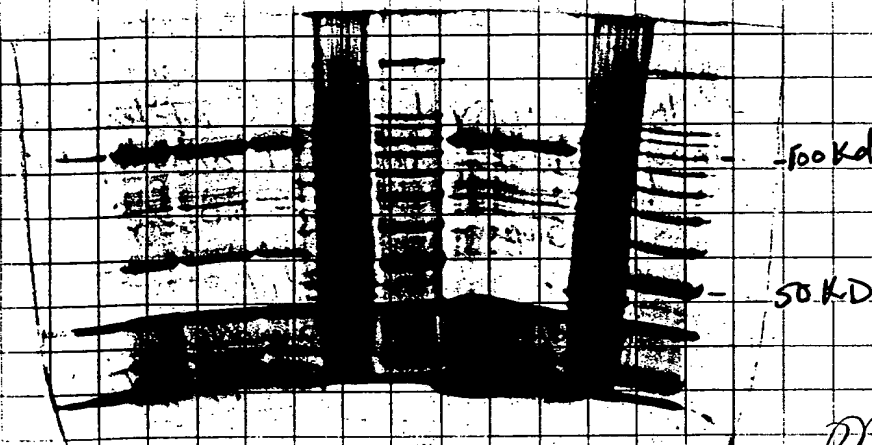
TITLE P41 + 3'-5' exo mutant SDS PAGE

From Page No. \_\_\_\_\_

06/11

12% SDS PAGE.

|   |                          |           |           |                         |   |                                   |           |                             |    |
|---|--------------------------|-----------|-----------|-------------------------|---|-----------------------------------|-----------|-----------------------------|----|
| 1 | 2                        | 3         | 4         | 5                       | 6 | 7                                 | 8         | 9                           | 10 |
| 1 | 6 $\mu$ g                | 4 $\mu$ g | 2 $\mu$ g | 60 $\mu$ g              | M | 4 $\mu$ g                         | 2 $\mu$ g | 60 $\mu$ g                  | M  |
|   | FYI<br>post heat<br>kill |           |           | FYI<br>pre heat<br>kill |   | 3'-5' exo M.<br>post heat<br>kill |           | pre heat<br>kill 3'-5' exo- |    |



DRY 6/20/95  
STF  
C

pre heat kill - spun down sup loaded on gel

To Page 1

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Date

Invented by

Date

May Longo

6/20/95

Record d by

06/16/95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

22

From Page No. \_\_\_\_\_

3/12/95

① 1 kb ladder ② T.nra/SPORT uncut, ③ Sst, ④ Sst/Sph, ⑤ Sph, ⑥ 1 kb ladder. (from 263 added loading dye, electrophoresis @ 190 V

- ~~digested~~ double digested BamHI/SphI (to map the Bam site T.nra)

H<sub>2</sub>O - 14.0  $\mu$ l

control: - H<sub>2</sub>O - 14.0  $\mu$ l

(REACT) buffer - 2.0  $\mu$ l

(uncut) buffer - 2.0  $\mu$ l

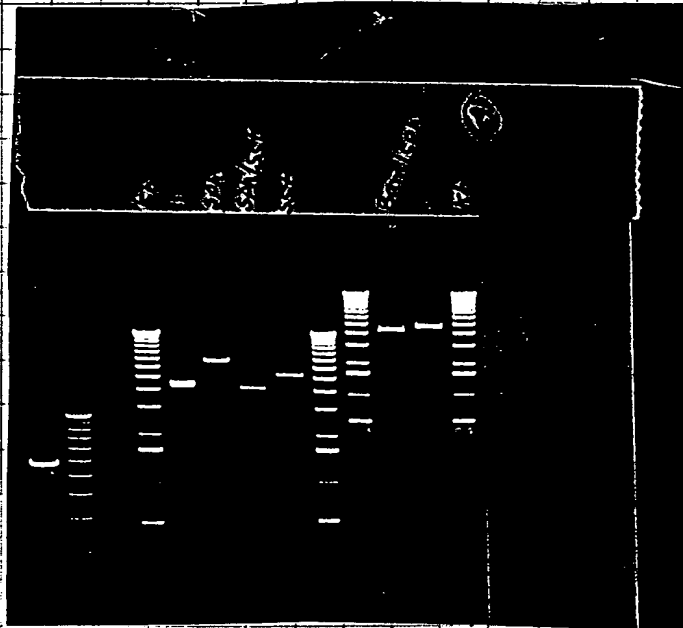
100 kb (T.nra/SPORT) DNA - 2.0  $\mu$ l

DNA - 2.0  $\mu$ l

(Bam/Sph) enzyme - 1.0  $\mu$ l ea.

TV = 20.0  $\mu$ l.

Incubated @ 37°C for 30 min. (15 min.)



To Page 1

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*[Signature]*

Date

4/12/95

Invented by

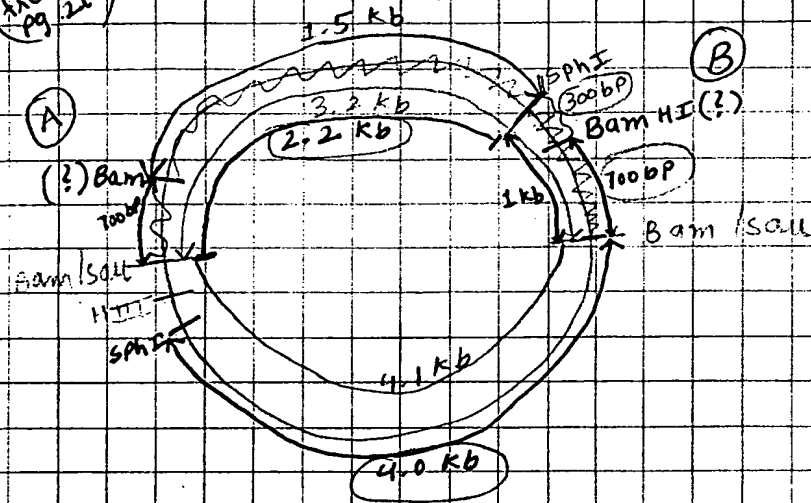
R c r d by *[Signature]*

Date

4/12/95

Page No. (from pg. 21)

GRAPH 2:



*Bam* / *Sph*

(A)

4.0 Kb  
700 bp  
1 Kb  
1.5 Kb

*Bam* / *Sph*

(B)

4.0 Kb  
2.2 Kb  
300 bp  
700 bp

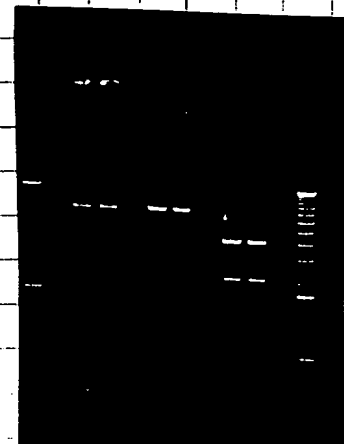
3.2  
1.7  
1.5

Cloning mp 18 w/ T. nea / pSPORT & mp 19 w/ T. nea / pSPORT

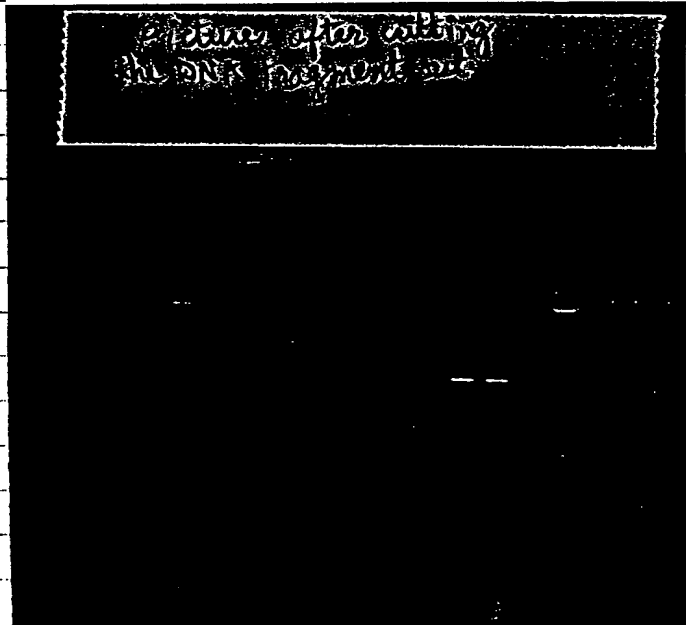
3/2/95

3/2/95

Thurs.



Picture before cutting the DNA fragment.



DID GENE CLEAN.

To Page N \_\_\_\_\_

ss d & Understood by me,

*[Signature]*

Date

4/12/95

Invent d by

Recorded by

*[Signature]*

Date

4/12/95

Project No. \_\_\_\_\_  
Book No. \_\_\_\_\_ TITLE PMC9 / Tag / Tag + DV / F+  
122 12/6/94

From Page No. \_\_\_\_\_

Purpose: - To check these primers with 2V of enzy.  
+ 3 step cycle.

- Repeat of expt. page 116 - 118 but used

non dv forward & reverse  
47.3  $\mu$ M 52.9

| 12x of each with Tag or Tag + D.V |       |       |             |
|-----------------------------------|-------|-------|-------------|
| 10x buffer                        | 499.2 | 499.4 | 200 $\mu$ M |
| dNTP                              | 60    | 60.0  | 200 $\mu$ M |
|                                   | 12    | 12.0  | 1 $\mu$ M   |
| Temp                              | 2.4   | 2.4   | K.T. buff   |
| primer 2                          | 11.5  | 11.5  | enzy        |
| 1                                 | 12.7  | 12.7  | Tag + DV    |
| enzyme                            | 4.8   | 12.0  |             |

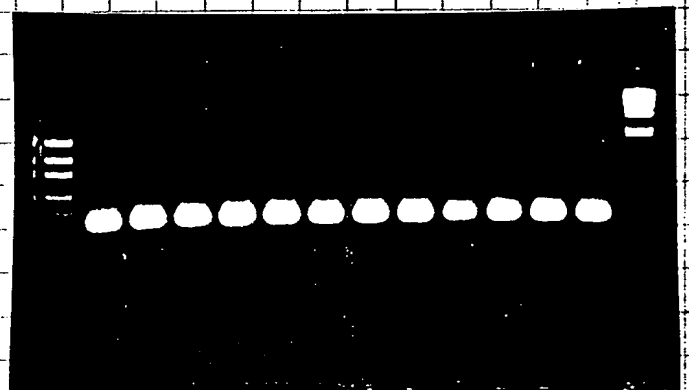
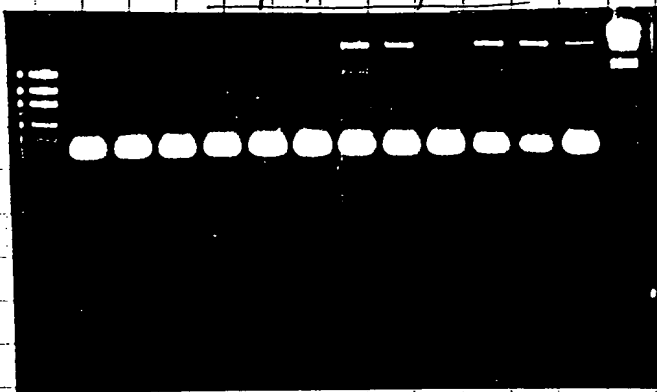
45  $\mu$ l of cocktail + 5  $\mu$ l of Mg different conc. 1 - 3 mM

Cycles 94°, 3'

30( 94°, 30", 56°, 30", 72°, 3' )

72°, 10"  $\rightarrow$  4° reheat  
Tag + Deep vent

Tag alone samples done



**age No.\_\_\_\_**

Result: Tag alone / 3 step cycle / F + R no-de didn't work  
again.

Taf + D.V : as usual even with 10 2 mm - 3 mm coats.

for same reason these new primers at 1  $\mu$ M conc./50  $\mu$ l gives hot  $\phi$  primer dimers.

Judys' 2 step cycle works with Tag 2U

Not transformed anything yet

**To Page No.\_\_\_\_\_**

~~sed & Underst~~ od by m ,

**Date**

**Invent d by**

**Date**

**Recorded by**

Iy. Sitarawan

LA 8794

| 86 min |     | Project No. <u>06</u> | Book No. <u>Turnover</u> | TITLE   | Amount         |
|--------|-----|-----------------------|--------------------------|---------|----------------|
| 01     |     | 543.00                | BK60                     | (14)    | 61 269.00      |
| 02     |     | 650.00                | 110                      |         | 62 7412.00     |
| 03     |     | 1014.00               | 476                      | 24      | 63 16953.00    |
| 04     | V   | 1485.00               | 771                      | 22      | 64V 36825.00   |
| 05     |     | 2627.00               | 2148                     | 34      | 65 44610.00    |
| 06     |     | 3187.00               | 2725                     | 32      | 66 62771.00    |
| 07     |     | 525.00                | BK60                     |         | 67 241.00      |
| 08     |     | 662.00                | 141                      | 30      | 68 3518.00     |
| 09     | DN  | 948.00                | 436                      | 33      | 69 9506.00     |
| 10     |     | 1271.00               | 769                      | 33      | 70DN 17320.00  |
| 11     |     | 1677.00               | 1188                     | 34      | 71 25050.00    |
| 12     |     | 2340.00               | 1871                     | 42      | 72 28643.00    |
| 13     |     | 624.00                | BK60                     |         | 73 324.00      |
| 14     |     | 694.00                | 72                       | (32)    | 74 1974.00     |
| 15     | Tne | 796.00                | 177                      | 27      | 75 5340.00     |
| 16     |     | 880.00                | 264                      | 23      | 76Tne 9478.00  |
| 17     |     | 976.00                | 363                      | 22      | 77 13880.00    |
| 18     |     | 1110.00               | 501                      | 22      | 78 19753.00    |
| 19     |     | 805.00                | BK60                     | 785 Ave | 79 321.00      |
| 20     |     | 977.00                | 192                      | 25      | 80 8826.00     |
| 21     | V   | 1409.00               | 467                      | 23      | 81 23029.00    |
| 22     |     | 1803.00               | 762                      | 23      | 82V 37324.00   |
| 23     |     | 2832.00               | 1133                     | 32      | 83 47661.00    |
| 24     |     | 3299.00               | 1883                     | 31      | 84 61758.00    |
| 25     |     | 774.00                | BK60                     |         | 85 404.00      |
| 26     |     | 918.00                | 99                       | 25      | 86 4493.00     |
| 27     | DN  | 1406.00               | 415                      | 36      | 87DN 12238.00  |
| 28     |     | 2277.00               | 1118                     | 44      | 88 21497.00    |
| 29     |     | 2989.00               | 1651                     | 45      | 89 30491.00    |
| 30     |     | 4085.00               | 2472                     | 50      | 90 36800.00    |
| 31     |     | 777.00                | BK60                     |         | 91 214.00      |
| 32     |     | 813.00                | 21                       | (12)    | 92 2257.00     |
| 33     | Tne | 947.00                | 121                      | 21      | 93Tne 6671.00  |
| 34     |     | 1136.00               | 263                      | 24      | 94 12685.00    |
| 35     |     | 1204.00               | 314                      | 19      | 95 19429.00    |
| 36     |     | 1631.00               | 633                      | 26      | 96 27534.00    |
| 37     |     | 919.00                | BK60                     | 922 Ave | 97 239.00      |
| 38     |     | 1284.00               | 251                      | 36      | 98 7128.00     |
| 39     |     | 1754.00               | 530                      | 35      | 99V 17335.00   |
| 40     | V   | 2728.00               | 1150                     | 39      | 100 32171.00   |
| 41     |     | 3910.00               | 1903                     | 42      | 101 45795.00   |
| 42     |     | 5168.00               | 2704                     | 46      | 102 56065.00   |
| 43     |     | 924.00                | BK60                     |         | 103 318.00     |
| 44     |     | 1205.00               | 180                      | 41      | 104 4474.00    |
| 45     | DN  | 1892.00               | 617                      | 48      | 105DN 11839.00 |
| 46     |     | 3234.00               | 1472                     | 57      | 106 19756.00   |
| 47     |     | 4572.00               | 2325                     | 58      | 107 29674.00   |
| 48     |     | 6365.00               | 3467                     | 62      | 108 36540.00   |
| 49     |     | 863.00                | BK60                     |         | 109 261.00     |
| 50     |     | 901.00                | 20                       | (7)     | 110 1566.00    |
| 51     | Tne | 953.00                | 103                      | 17      | 111Tne 4647.00 |
| 52     |     | 1083.00               | 103                      | 13      | 112 8879.00    |
| 53     |     | 1085.00               | 386                      | 27      | 113 12496.00   |
| 54     |     | 1529.00               | 92                       | 29      | 114 18327.00   |
| 55     |     | 984.00                | BK60                     |         | 115 295.00     |
| 56     |     | 891.00                | 124                      | 18      | 116 1709.00    |
| 57     | Tne | 1067.00               | 92                       | 29      | 117 4261.00    |
| 58     |     | 1086.00               | 264                      | 18      | 118Tne 8343.00 |
| 59     |     | 1336.00               | 364                      | 25      | 119 12504.00   |
| 60     |     | 1467.00               | 347                      | 25      | 120 18443.00   |

Win

Deanna Polanco

Date 11/29/94

Inv nted by [Signature]  
Record d by

11-9-94

g N \_\_\_\_\_

JAMP BK60<sup>g</sup>

1. Cheng mix = 564 ave
2. Klentay mix = 785
3. Vent mix = 922

spot

Cheng

Klentay

Vent

$$75821 \text{ cpm} \left( \frac{50 \mu\text{l Rxn Vol}}{2 \lambda \text{ spotted}} \right) \left( \frac{200}{155} \right) \left( \frac{1}{2500 \mu\text{l}} \right) \left( \frac{1}{4} \right) = 194 \text{ cpm at } \mu\text{mol}$$

$$(267 \text{ cpm} / \mu\text{mol})$$

$$(314 \text{ cpm} / \mu\text{mol})$$

$\mu\text{mol incorp} =$   
(200  $\mu\text{l Rxn}$ )

$$\frac{\text{cpm}}{\text{cpm} / \mu\text{mol}} \left( \frac{200}{155} \right) \left( \frac{20}{15} \right)$$

$\mu\text{mol turnover} =$   
200  $\mu\text{l Rxn}$

$$\frac{\text{cpm} - \text{BK60}}{\text{cpm} / \mu\text{mol}} \left( \frac{200}{155} \right) \left( \frac{10}{2} \right)$$

$$\% \text{ turnover} = \frac{\mu\text{mol turnover}}{\mu\text{mol turnover} + \mu\text{mol incorp}}$$

121 75821.00  
122 104512.00

To Page No. \_\_\_\_\_

Read & Understood by me,

Michael Polay

Date

11/29/94

Invented by

Recorded by

Date

11-10-94

DEI +  $(\text{NH}_4)_2\text{SO}_4$  ppt.

Project No. \_\_\_\_\_  
B k No. \_\_\_\_\_

06/15/98

in to continue with purification - following the  
the protocol as in wild type Tne - p.108.

11 - 6.8 mL  $(.05)(6.8) = 2M \times$   $x = 174 \mu\text{L}$  of 2M KCl

exo - 4.8 mL 3.8  $(.05)(4.8+x) = 2M \times$   $x = 97.4 \mu\text{L}$  of 2M KCl

$(.40)(6.8+x) = 10\% \times$   
 $291 \mu\text{L} = x$   $x = 291 \mu\text{L}$  10% DEI

$(.40)(3.9+x) = 10\% \times$   
 $143 \mu\text{L} = x$   $x = 143 \mu\text{L}$  10% DEI

Make each a Anal 50mM KCl slowly add 20% a 10%  
DEI sol'n to a Anal [3] of .4%. vortex - let shake  
30 minutes @ 4°C. spin in 2mL eppendorf in micro-  
centrifuge 20 minutes @ 4°C - Save Supernatant.

60%  $(\text{NH}_4)_2\text{SO}_4$  fractionation

TX1  $\frac{36g \text{ solid}}{100 \text{ mL}} = \frac{x}{4.8 \text{ mL}}$  2.45g

3'-5' exo-  $\frac{36g}{100 \text{ mL}} = \frac{x}{3.5 \text{ mL}}$  1.26g

vortex - let shake 30min @ 4°C  
spin in 55-34 - 20,000 x g -  
Decant + Save Supernatant - Pellet's

To Page No. \_\_\_\_\_

|                                      |                 |                            |                  |
|--------------------------------------|-----------------|----------------------------|------------------|
| sed & Understood by me,<br>May 20/98 | Date<br>6/20/98 | Invented by<br>E. H. H. H. | Date<br>06/16/98 |
|                                      |                 | Recorded by                |                  |

From Page No. \_\_\_\_\_

Bump Heparin with .5M NaOH - Wash w/ H<sub>2</sub>O extensive  
 Equilibrate w/ Buffer A.

Buffer A - Heparin -

Buffer B - Heparin

25mM Tris pH 7.4

10% glycerol

5mM Bme

.1mM PMSF

.1mM EDTA

10mM KCl

conductivity - 1.2mS

A.S.

25mM Tris pH 7.4

10% glycerol

5mM Bme

.1mM PMSF

.1mM EDTA

1.5M KCl

TY-1 - Dissolve Pellet in 10mL of Buffer A

4.5mS - conduct

Add 30mL additional of Buffer A

2.1mS - conduct

Load 9 35mL on 2mL TBSO Heparin @ .75mL/min  
 collect flow through material - wash to base line -

Gradient Program - 0 - 100% B @ .5mL/min - 20mL linear  
 wash 100% B - 10mL - @ .5mL/min  
 collect 500  $\mu$  fractions -

To Page

Witnessed &amp; Understood by me,

Date

Inv. nted by

Date

Recorded by

M. J. Jones

6/20/55

6/15/95

6/15/95

ag N \_\_\_\_\_  
Mtx Rxn

tock

For 20 mL

SMTAPS

1 mL

50 mM  $MgCl_2$ 800  $\mu$ L

2M KCl

500  $\mu$ L

1 M DTT

200  $\mu$ L

10 mM dNTPs

400  $\mu$ L

ct. Salmon testes

5 mL

12.1

- 1.1 mL dCTP  
vial

20 mLs

Aliquant 500  $\mu$ L / tube store in  $-20^{\circ}\text{C}$  freezer - yellow tubes -

To Page No. \_\_\_\_\_

Read &amp; Understood by me,

Date

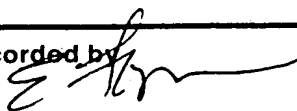
Invented by

Date

Way Long

6/20/95

Recorded by



6/16/95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

Heparin - FY-1

142

From Page No. \_\_\_\_\_

06/15

SAM

CPM1

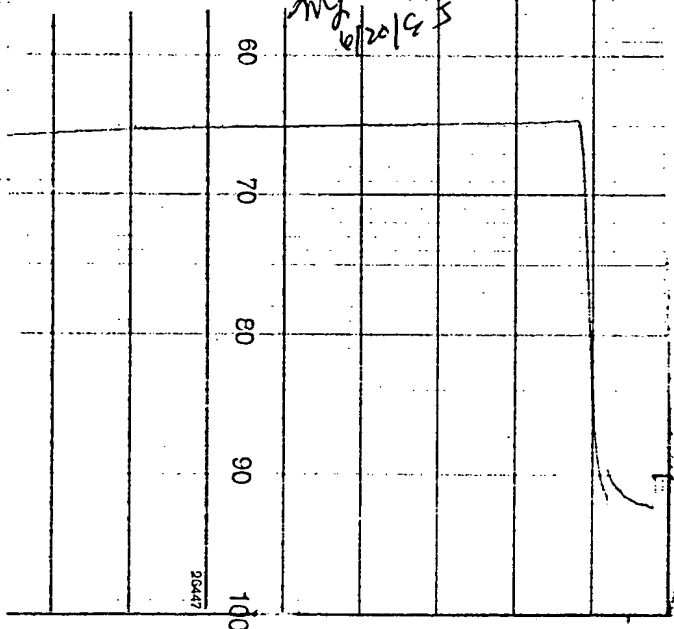
EX

FY-1

|    |             |
|----|-------------|
| 1  | 115552.0052 |
| 2  | 53328.0054  |
| 3  | 9146.0056   |
| 4  | 4556.0058   |
| 5  | 1260.0059   |
| 6  | 3744.0060   |
| 7  | 1028.0061   |
| 8  | 574.0062    |
| 9  | 536.0063    |
| 10 | 346.0064    |
| 11 | 730.0065    |
| 12 | 438.0066    |
| 13 | 348.0067    |
| 14 | 21268.0068  |
| 15 | 668.0069    |
| 16 | 372.0070    |
| 17 | 866.0071    |
| 18 | 74836.0072  |
| 19 | 146.00      |

Pool 47-55 dialyze o/n in Queso Buffer A

my 6/20/95



Pharmacia LKB Biotechnology

2.4 µl Rxn  
 1 µl pack  
 Sample -  
 incubate @  
 in 8' - qu  
 w/ 10 µl of S  
 EDTA - SP  
 20 µl on 6  
 wash

5' 1x 10' TCI

3' 3x 5' T.

2x S to

dry + cou.  
 econoflow

Pool - 49

dialyze o/  
 in again  
 Queso Buf  
 See p. 144

11/11/95

To Page N

With ss d &amp; Understood by me,

Date

Invented by

Date

R corded by

Mar Torgo

4/20/95

S. Kym

06/16/95

Hepairn 3-5 cno mutant

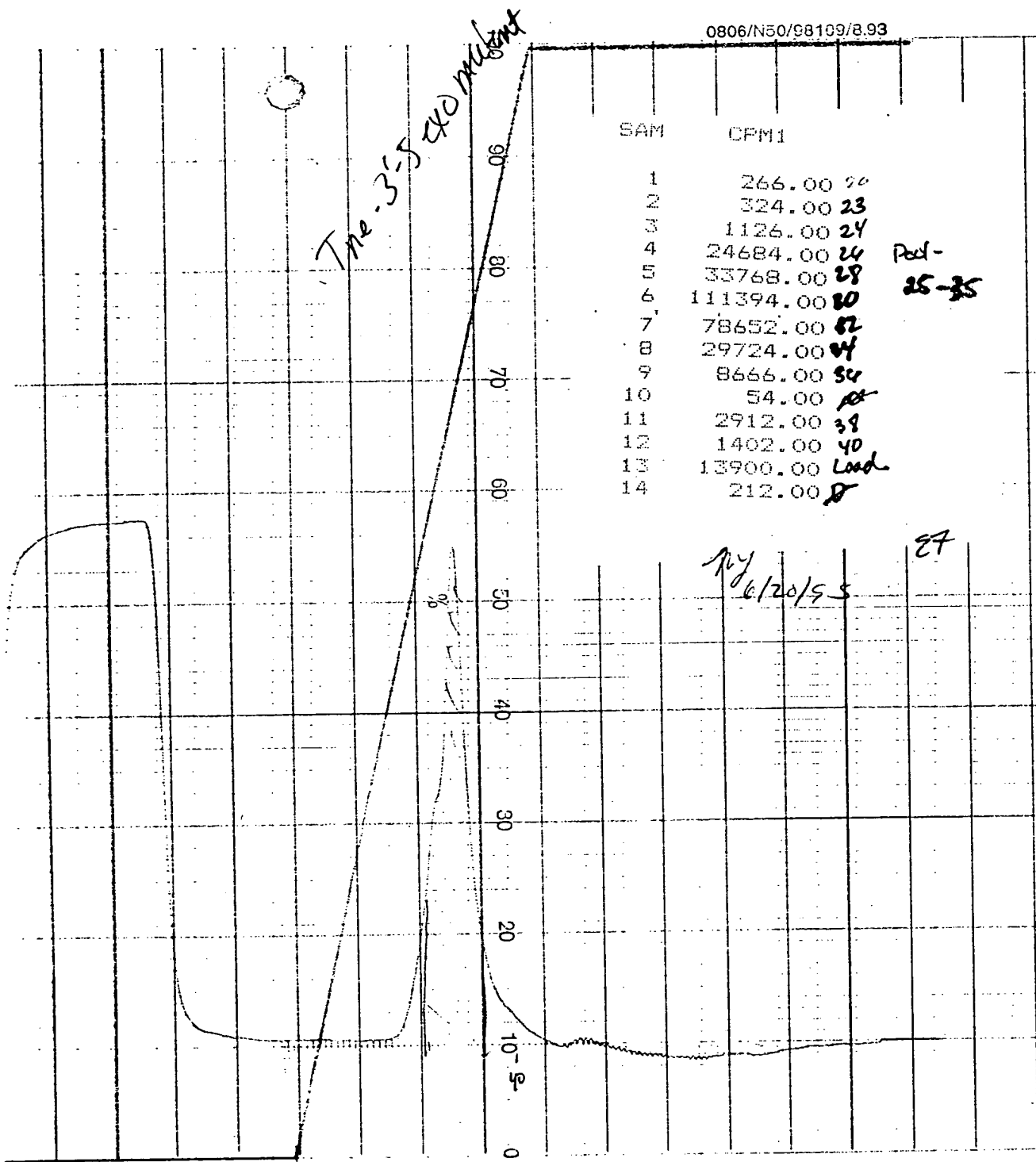
Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

143

ag No. \_\_\_\_\_

06/15



24  $\mu$ l mix  
1  $\mu$ l fraction  
Sample -  
incubate @  
74° 8 min -  
quench w/  
10  $\mu$ l g. SM  
EDTA -  
Spot 20  $\mu$ l  
on GF/C  
wash -  
1x 10% TCA  
1x PC

3x 5% TCA  
2x EtOH  
dry +  
count -

Pool - 25-35  
dialyze 4 hrs  
in QLSO  
Buffer A  $\rightarrow$   
See p. 148

5/15/95

Technology

Code No. 18-1001-44

To Page No. \_\_\_\_\_

sed & Understood by me,

Date

Invented by

Date

Recorded by

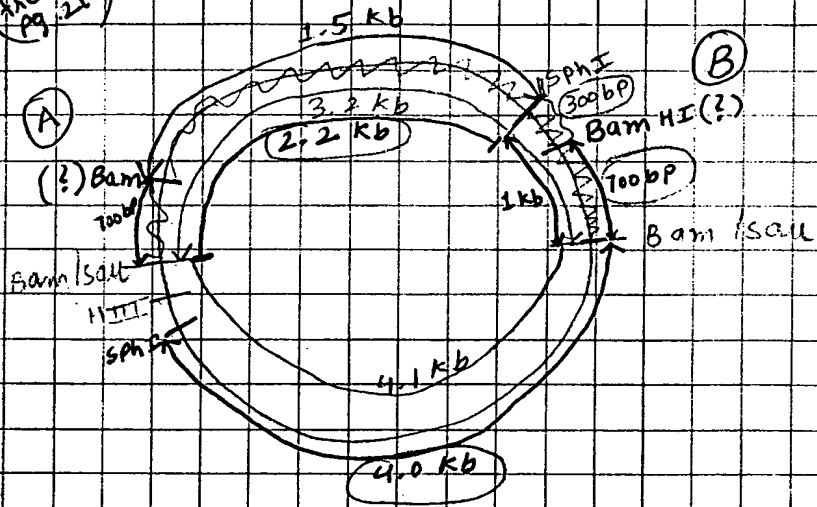
May 16/95

6/20/95

El. Zank

06/16/95

ag No. (X100M  
Pg. 21)  
RAPH 2:



*Bam*/Sph

(A)  
4.0 kb  
700 bp  
1 kb  
1.5 kb

*Bam*/Sph

(B)  
4.0 kb  
2.2 kb  
300 bp  
700 bp

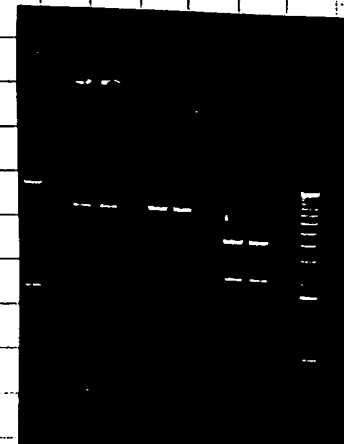
3.2  
1.7  
1.5

Cloning mp 18 w/ T.nea/psport & mp 19 w/  
T.nea/psport

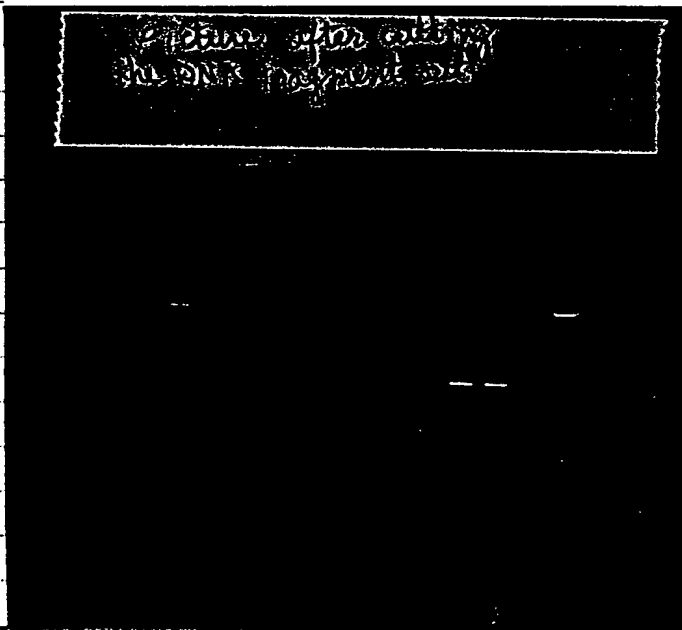
3/2/95

3/2/95

Thurs.



Picture before  
cutting the  
DNA  
fragment.



DID GENE CLEAN.

To Page No. \_\_\_\_\_

sd & Und rstood by me,

Date

4/14/95

Invented by

Recorded by

Date

4/12/95

From Page No. \_\_\_\_\_

GENE CLEAN

- Mixed mp 18 with T-neo pSPORT cut w/ Sst I sph } 1 tube
- " mp 19 with " " " " " } 2 tube

- added 700.0  $\mu$ l NaI to each 2 tubes. Vortexed
- put the tubes in 55°C heat block to melt agarose
- after agarose melted, added 5.0  $\mu$ l glass milk to both tubes
- incubated both tubes on ice for 5.0 min.
- cfg. both tubes (quick spin)
- discarded supernate & washed pellet 3 x with New Wash 6
- added 14.0  $\mu$ l dH<sub>2</sub>O to each tube
- quick spinned, discarded pellet & saved supernate.

Set-up Ligation

- (mp 18) (mp 19)  
DNA - 14.0  $\mu$ l
- (ligase) 5x buffer - 4.0  $\mu$ l
- ligation - 2.0  $\mu$ l.
- TV - 20.0  $\mu$ l.

- incubated both

Transformation Cells

- (1) 100.0  $\mu$ l Competent
- 3.0  $\mu$ l DNA (from ligation)

- (2) incubated on ice for 30 min.

- (3) heat shocked @ 42°C H<sub>2</sub>O bath for 35 sec.

- melted 0.7% 2x YT top agar, added 4.0  $\mu$ l to 6 different glass tubes & put the tubes @ 55°C heat block

Did not work

T Pag No

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Dat

4/12/95

Inv nt d by

R c rded by

Dat

4/12/95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

124 12/6/94

From Page No. \_\_\_\_\_

Purpose: To try the new scheme! in 8 days!! 10 hrs  
18 hands!!!

I Vector prep: pvc 19 - 100  $\mu$ y \*  
0.45  $\mu$ y/l peeled from several tubes

- extracted with Aak II (1) - 1 hr = 8 hr  
ECORI & Bam HI (2) - 1 hr = 3  
Afl III (3) 2 hr = 2  
successively at 37°

LT1 pvc 19 100  $\mu$ y 225  $\mu$ l  
NEB Buffer 4 (10x) 40  
NEB Aak II (240 U/l) 10  
TE 125

5  $\mu$ y added  
1.25  $\mu$ y loaded

400 → 20  $\mu$ l saved after 1 hr at:

LT1 Aak II 403 EORI } 20  
74121 Bam HI } 20  
(0.6 U/l)

440

4.5  $\mu$ y  
1.075  $\mu$ y

LT1 Afl 3 & D1 (7403) } 30  
BMB 101 }

5' A' C P u r y G T-3'  
3' T G P y p u c A-5'

470

90  $\mu$ y

2 hr at 37°

Run a, b, c on minigel along with uncut pvc and  
carrier / Hind III marker.

5  $\mu$ l of a, b & c, uncut pvc 0.5  $\mu$ l = 0.225  $\mu$ y

Final (C) stored at 4° overnight.

To Page N

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Date

12/10/94

Invent d by

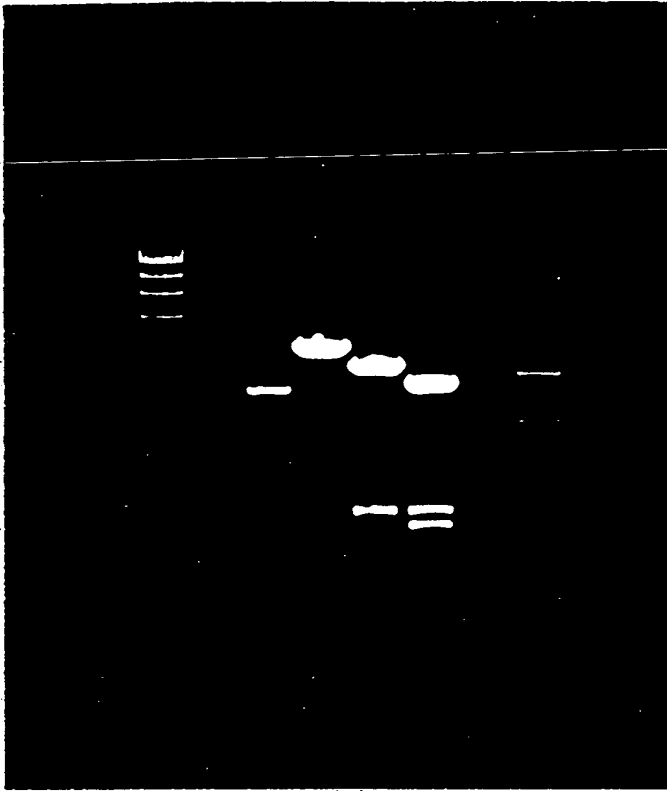
Recorded by

K. S. S. S. S.

Date

12/11/94

Page No. \_\_\_\_\_



unclut<sup>+</sup> Aat<sup>+</sup> EcoRI  
puc<sup>+</sup> BamHI Apl 3.

puc = 2686 bp

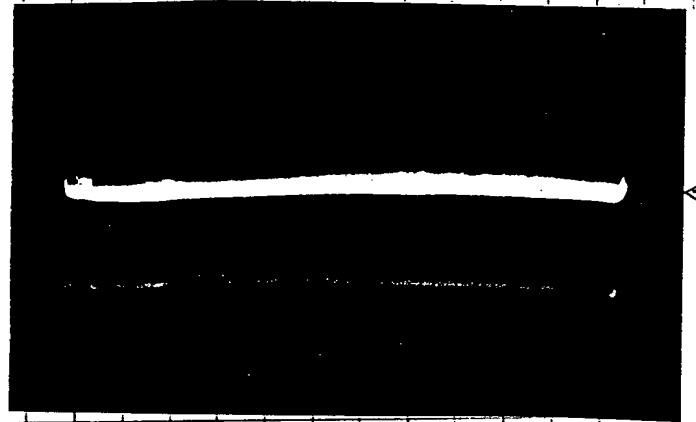
Aat II @ 2617 + Apl III @ 806 = Vector = 1811 bp 0.6742 / 1  
cut = 875 bp 0.325

DEAE paper containing the vector band, washed in { 1M NaCl  
for 10 mM Tris 8.  
5 mM EDTA

ethanol apt (Tol) "en" = 100 ng / 1

- Digestion seems to be complete

- All enzymes are potent!



- loaded as 40 µg of cut plasmid 19  
and purified the vector band  
- is 26.97 µg.

To Page No. \_\_\_\_\_

Issued & Understood by me,

Date

12/19/94

Invented by

Recorded by

Dr. Srinivasan

Date

12/7/94

126 12/7/94

Project No. \_\_\_\_\_  
B ok No. \_\_\_\_\_

TITLE puc / F&R non du transform

From Page N \_\_\_\_\_

Prep: To transform puc / F&R non du primers.

- amplified, ethanol pptd, cut with Dat II again  
linearized with Dat II & ligated
- 2<sup>5</sup> µl of ligation mix transformed with DH5α max if cells.
- Plated 25, 50, 100 µl from each reaction (from 5

Treated 4 different Rx: all prepared by Judy and

|   |                               |     |     |     |                      |
|---|-------------------------------|-----|-----|-----|----------------------|
| ① | Tag, 1.5 mM Mg<br>(395)       | 25  | 17  | -   | all blue<br>no white |
|   |                               | 50  | 9   | -   |                      |
|   |                               | 100 | 17  | -   |                      |
|   |                               |     |     |     |                      |
| ② | Tag, 2 mM Mg<br>(396)         | 25  | 2   | -   | ?                    |
|   |                               | 50  | 1   | -   |                      |
|   |                               | 100 | 3   | +   |                      |
|   |                               |     |     |     |                      |
| ③ | Tag + D.V. 1.5 mM Mg<br>(403) | 25  | 10  | 6   | 60%                  |
|   |                               | 50  | 25  | 17  | 68%                  |
|   |                               | 100 | 24  | 13  | 54%                  |
|   |                               |     |     |     | 60%                  |
| ④ | Tag + D.V. 2 mM Mg<br>(404)   | 25  | 136 | 40  | 29%                  |
|   |                               | 50  | 223 | 82  | 37%                  |
|   |                               | 100 | 405 | 141 | 35%                  |
|   |                               |     |     |     | 34%                  |

Result: Tag + D.V. unusually high error - mutation

Tag alone \* of colonies too low, but whatever is there not much white though.

nothing makes sense

To Page N \_\_\_\_\_

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

A. Johnson

12/8/94

Project No. \_\_\_\_\_ TITLE Repeat unit assay Qc for v12  
Book No. \_\_\_\_\_ lot # EKBT1 done on P 61

From Page No. \_\_\_\_\_  
lot # EKBT1 is ~ 401 u/ml based on P.61  
amplitude lot # 9957 for control

1. starting dilutions of EKBT1:

|                              |                                |
|------------------------------|--------------------------------|
| <u>1:80</u> (estimate cf=5%) | <u>1:160</u> (estimate cf=2.5) |
| lot EKBT1 5 µl               | 5 µl                           |
| Tag storage 395 µl           | 795 µl                         |
| buffer                       |                                |
| actual is 4.03% / 1          | actual is 2.01 %               |
| Vf = 400 µl                  | Vf = 800 µl                    |

2. 1/600 dilutions

| serial dilution # | 1-6                 | 7-12                  | 13-18 | 19-24 | 25-30 | 31-36 | 37-42 | 43-48 | 49-54 |
|-------------------|---------------------|-----------------------|-------|-------|-------|-------|-------|-------|-------|
|                   | I                   | II                    | III   | IV    | V     | VI    | A-1   | A-2   | A-3   |
| 1:80 dil          | 31                  | 3                     | 3     |       |       |       |       |       |       |
| 1:160 dil         |                     |                       |       | 3     | 3     | 3     |       |       |       |
| Amplitude 5%      |                     |                       |       |       |       |       | 3     | 3     | 3     |
| lot #             |                     |                       |       |       |       |       |       |       |       |
| dilution buffer   | 1797 µl             | use from 20 and 40 ml |       |       |       |       |       |       |       |
|                   | Vf = 2000 / 1800 µl |                       |       |       |       |       |       |       |       |

3. Serial dilutions

| serial dilutions # | 1      | 2      | 3      | 4      | 5      | 6         |
|--------------------|--------|--------|--------|--------|--------|-----------|
| dilution buffer    | 100 µl | 100 µl | 100 µl | 100 µl | 100 µl | 1 ml of I |
|                    | 300 µl | 300 µl | 300 µl | 300 µl | 300 µl |           |

dilute I - A-3 as shown for I below:

dilute I - III and assay  
then dilute IV - VI and assay  
then dilute A-1 - A-3 and assay

SA I-III = 45 µl assay mix + 5 µl dil buffer, do same for IV-VI  
spot 4x 5 µl on 6 FC in 4 quadrants  
Blank is 45 µl assay mix + 5 µl dil buffer → spot on GFC along with other

Page No. \_\_\_\_\_

# 55-57 = Blank for I-III, IV-VI and A1-A3 respectively

58-61 = SA for I-III

62-65 = SA for IV-VI

Result:

using amphotag lot #9957 here gives a  
 unit value of ~~320 u/pl~~ 323.4 u/pl  
 compared to 401 u/pl (found on P, 61, 10-1-94)

To Page No. \_\_\_\_\_

s d &amp; Und rstood by m ,

Date

Invented by

Date

Sandra Pokay

11/6/95

Recorded by

10-15-94

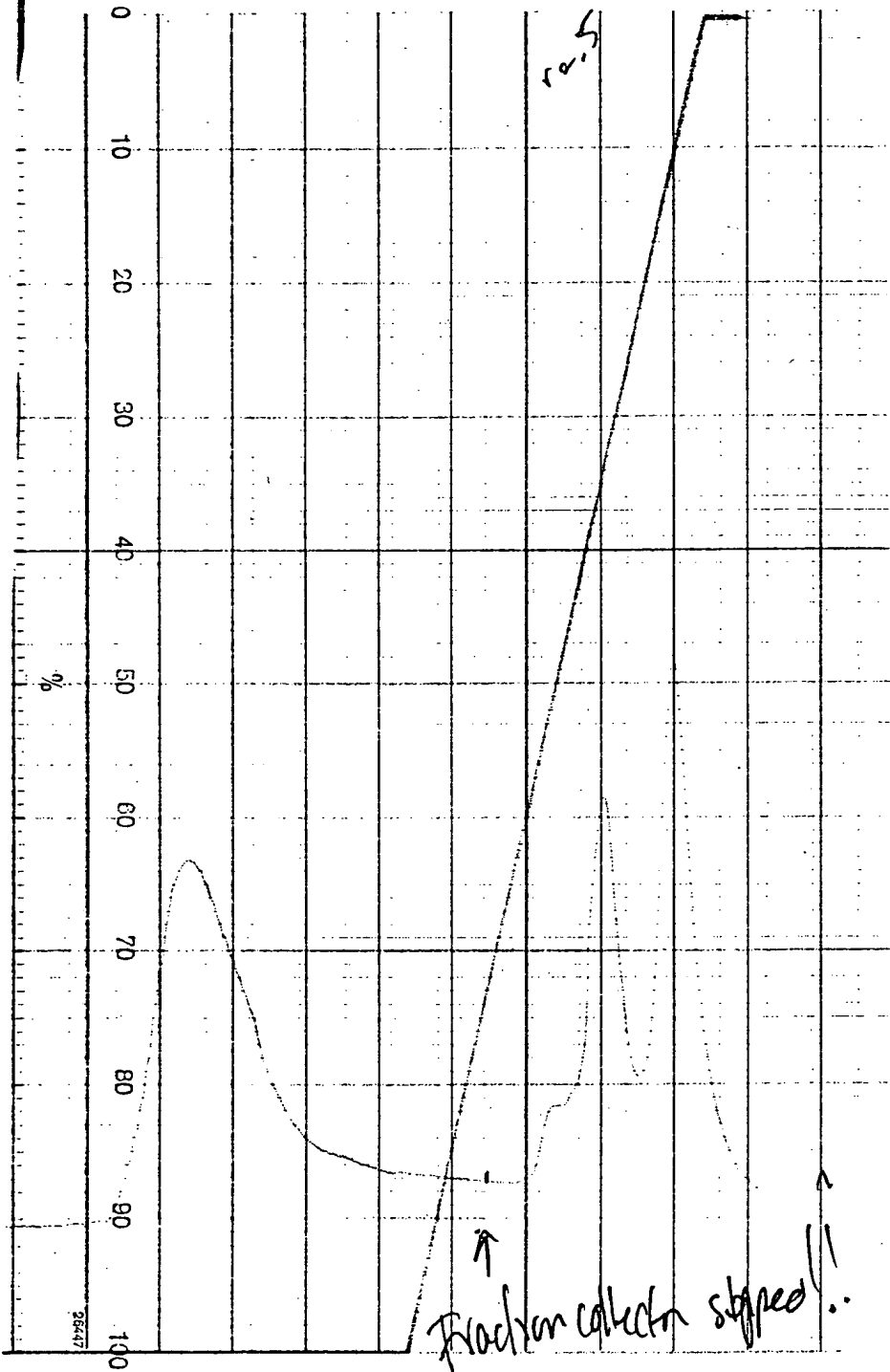
Project No. \_\_\_\_\_  
Book No. \_\_\_\_\_

TITLE FY-1 Q6SDM-2ml

146

From Page No. \_\_\_\_\_

06/11



Q Buffer A -

25mM Phos - pH 7.2  
.1mM EDTA  
10mM KCl  
5mM Bme  
10% glycerol

Q Buffer B

25mM Phos - pH 7.2  
.1mM EDTA  
800mM KCl  
5mM Bme  
10% glycerol

6/20/95

Pharmacia LKB Biotechnology

Code No. 18-100

To Page 1

Witnessed & Understood by me,

Date

Invented by

Date

May Longo

6/20/95

Recorded by

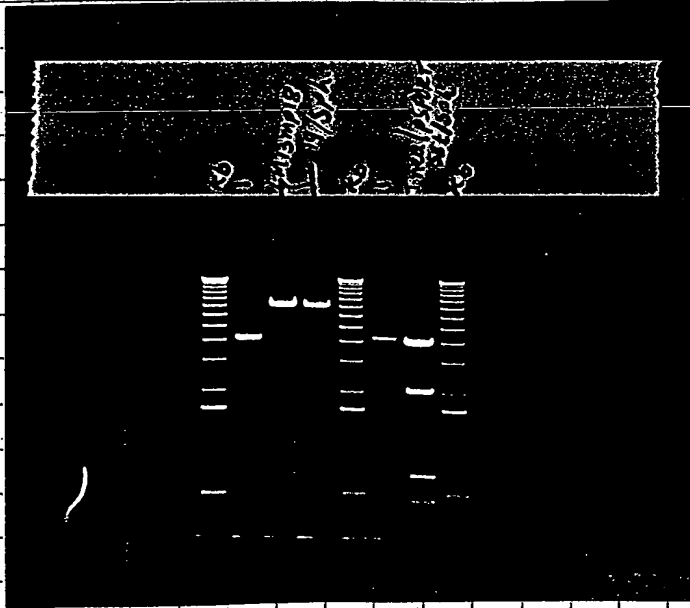
S. Hyman

06/16/95

ag No. \_\_\_\_\_

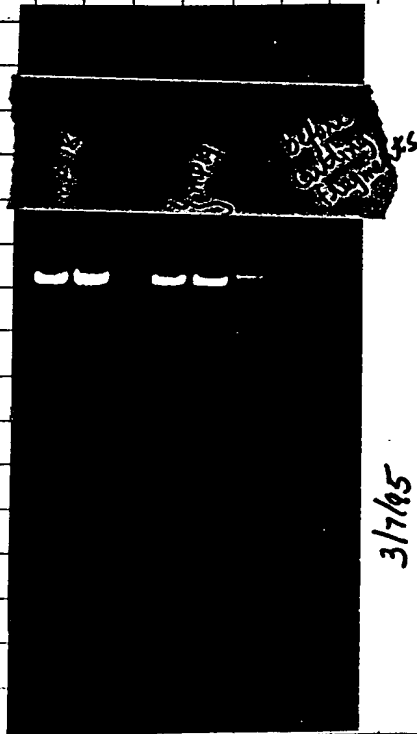
Repeat

3/7/95 TUE

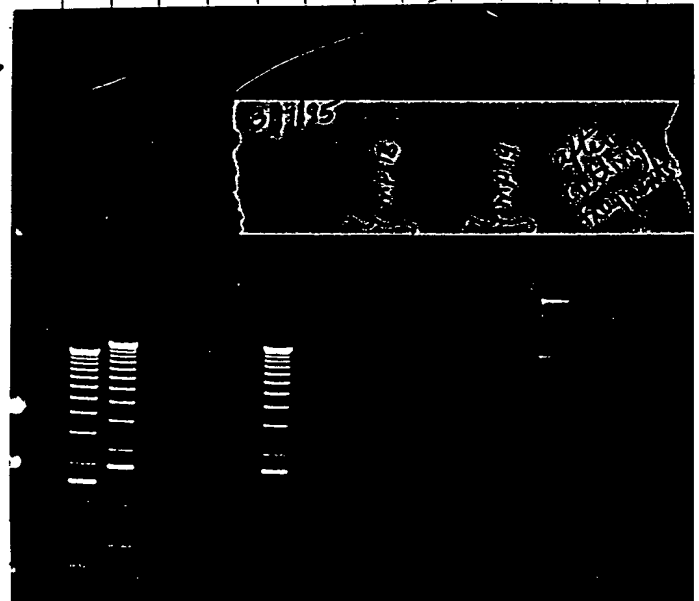


3/7/95

After taking picture on looking @ the gel, ~~M13~~ \* M13 mp 18 and M13 mp 19 is @ the 7.2 kb ~~cut~~ which was cut with Sph I. ~~we decided to cut~~  
 We planned on cutting mp 18 and mp 19 with Sst I. The gel <sup>picture</sup> below shows mp 18 & mp 19 before & after cutting the DNA fragments. After cutting the fragment performed Gene CLEAN



3/7/95



To Page No. \_\_\_\_\_

is d & Understood by me,

*Bokey*

Dat

4/12/95

Inv nt d by

R cord d by

*Shan*

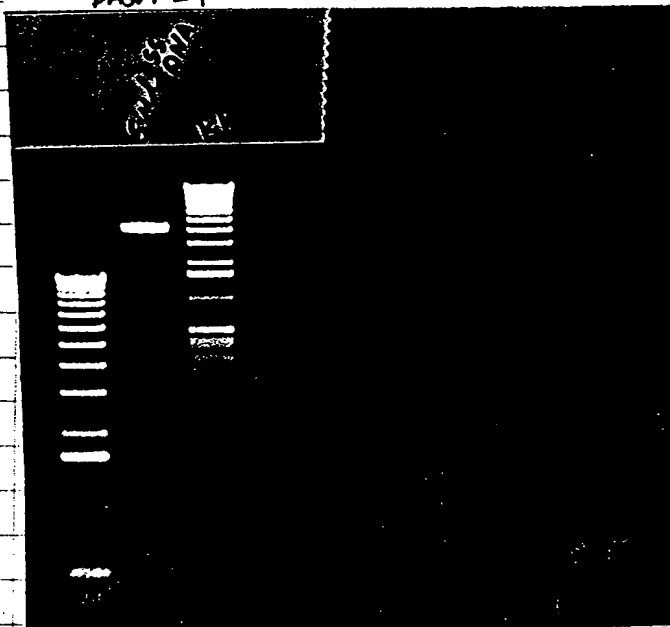
Date

4/12/95

From Page No. \_\_\_\_\_

labelled 2 tubes, 1 w/ mp 18, & 2<sup>nd</sup> w/ mp 19

1. to the DNA w/ agarose gel, added 100.0  $\mu$ l NaI
2. put the tubes @ 52°C heat block to melt agarose, vortexed constant
3. added 5.0  $\mu$ l glass milk to both tubes - mixed
4. incubated on ice for 5 min.
5. (fg. (quick spin) @ room temp.
6. discarded supernate, added 500.0  $\mu$ l New wash buffer
7. discarded supernate, washed pellet 3x with New wash buffer
8. after washing 3x, added 14.0  $\mu$ l dH<sub>2</sub>O to the pellet (discarded & (mixed)
9. incubated @ 52°C for 5 min.
10. discarded pellet & saved supernate for ligation.  
(could this on 3/8/95 wed.)

Purification of m13 ssDNA (T. res 2 kb [SphI] / mp 19) from pg. 1T. res (mp 19) ssDNA (SphI)  
DH5 $\alpha$  50amp  
4/12/95  
JR

To Page 1

Witnessed &amp; Understood by m ;

Date

4/12/95

Invented by

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Date

4/12/95

ag No. \_\_\_\_\_

cell growth & infection

- Grew an *E. coli* F' strain to an OD of 0.2-0.4 in 2x YT
- Inoculated 1-2 ml of the cells w/ the phage. (added 10.0  $\mu$ l from a liquid phage stock & added to cells)
- Incubated the phage infected cells @ 37°C for 5-7 hours.
- The supernate can now be processed for isolation of ssDNA & the cells can be processed for the isolation of Replication Form (RF) dsDNA.

Purification of ml3 ssDNA

- > transferred 1.0 ml culture of infected cell to 4 different eppendorf tubes
- cfg 4 tubes for 2 min.
- transferred supernate to the new tubes & saved pellet from 1 tube (out of 4 tubes) for isolation of RF DNA
- Spinned the supernate again & transferred the supernate to the new tubes (done to remove any residual cells remained behind)
- passed the supernate through a 0.45  $\mu$  filter as to remaining cells (done when performing site-directed mutagenesis)
- added 200.0  $\mu$ l of 20% PEG + 1.5 M NaCl. Vortexed
- Incubated tubes for 15 min @ room temperature (or overnight @ 4°C)
- cfg for 10 min in a 4cfg. @ room temp.
- discarded supernate & briefly spinned the tubes to remove the residual soln from the side of the tube (removed as much <sup>supernate</sup> as possible)
- added 200.0  $\mu$ l TE. Vortexed
- cfg for 2 min. to remove any residual cell debris.
- Transferred supernate to the new tube. (added 5.0  $\mu$ l RNase I to remove any residual nucleic acid from the prep. Benzonase will remove both RNA & DNA very efficiently.)
- added equal volume of phenol/chloroform/isoamyl alcohol. mixed well.
- cfg for 5.0 min.
- transferred the upper layer to a new tube (BE CAREFUL NOT TO DISTURB WHITE INTERFACE OR REMOVE ANY PHENOL)
- added 20.0  $\mu$ l NaAc & 600.0  $\mu$ l EtOH
- Incubated @ -70°C for 5-15 min. (we left @ -70°C overnight)

To Page N. \_\_\_\_\_

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Date

Invent d by

Date

JDB/10/10/95

4/12/95

Recorded by

4/12/95

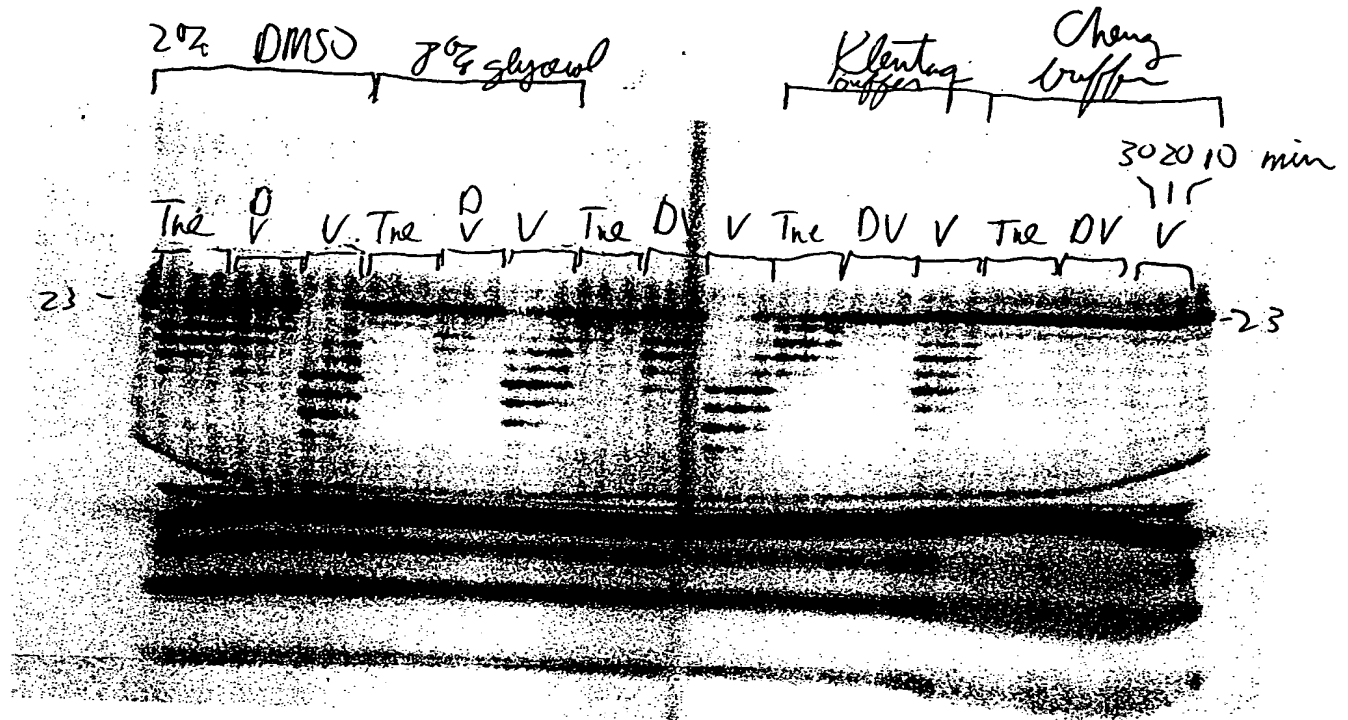
82

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

From Page No. \_\_\_\_\_



Result.

T Pag No.

Witnessed & Understood by me,  
Deena Polay

Date  
11/29/94

Invented by  
Recorded by

Date  
11-5-94

**From Page No.\_\_\_\_**

|                 | Chemical  | Concentration | Cloning | Electroporation | Transformation |
|-----------------|---|---------------|---------|-----------------|----------------|
| Chemical        | Tris-HCl  | pH 8.7        | 20 mM   | 50 mM           |                |
|                 | Tris-HCl  | pH 9.1        |         |                 |                |
|                 | K <sup>+</sup> DAc                              | pH 8.7        | 85      |                 | 20 mM          |
|                 | K <sup>+</sup> Cl                               |               |         |                 | 10             |
|                 | (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> |               | 1.2     | 1.6             | 10             |
| Cloning         | Mg(OAc) <sub>2</sub>                            |               | 1.2     |                 |                |
|                 | MgSO <sub>4</sub>                               |               | 2       | 1.2             | 2              |
|                 | DMSO  |               |         |                 |                |
|                 | Tris-HCl  |               |         |                 | 0.1%           |
|                 | Tris-HCl  |               |         |                 |                |
| Electroporation | Tris-HCl  |               |         |                 |                |
|                 | Tris-HCl  |               |         |                 |                |
|                 | Tris-HCl  |               |         |                 |                |
|                 | Tris-HCl  |               |         |                 |                |
|                 | Tris-HCl  |               |         |                 |                |

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|---|---------|
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**Nitin** ssed & Understood by me,

Deena Polay

**Date**

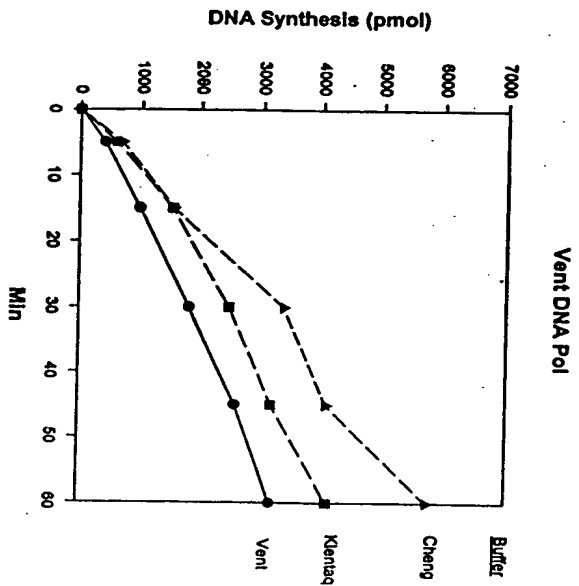
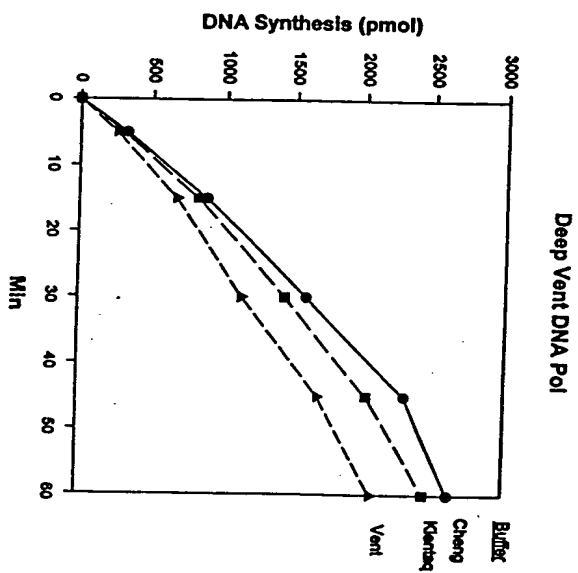
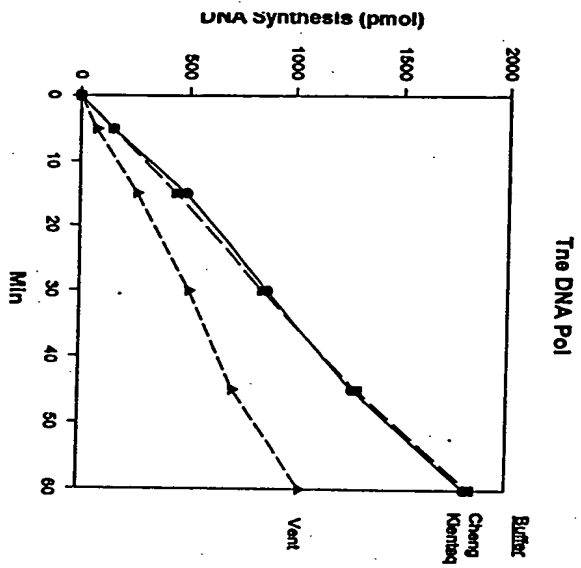
"129/94

**Invented by-**

Record d by

**Date**

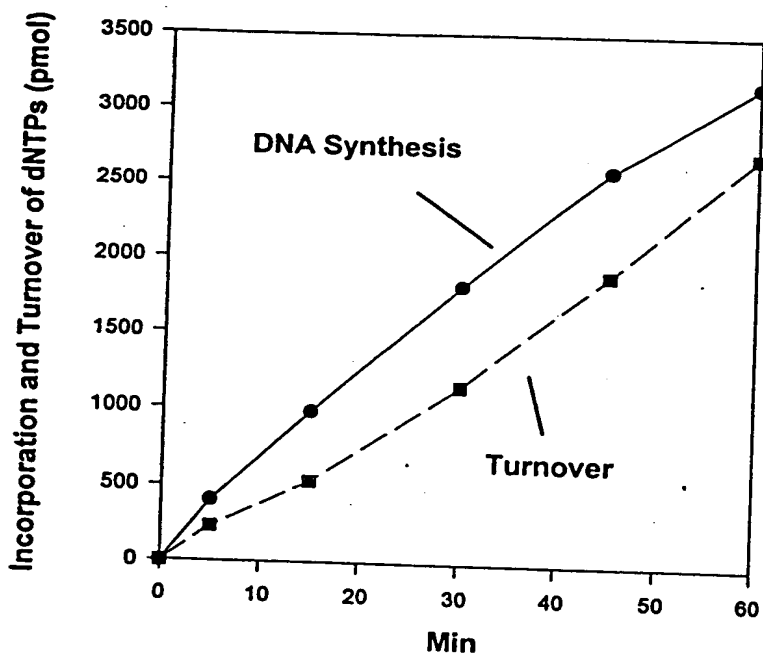
11-5-94



In each case, DNA synthesis is lower in  
 Primer degradation was highest in Vent

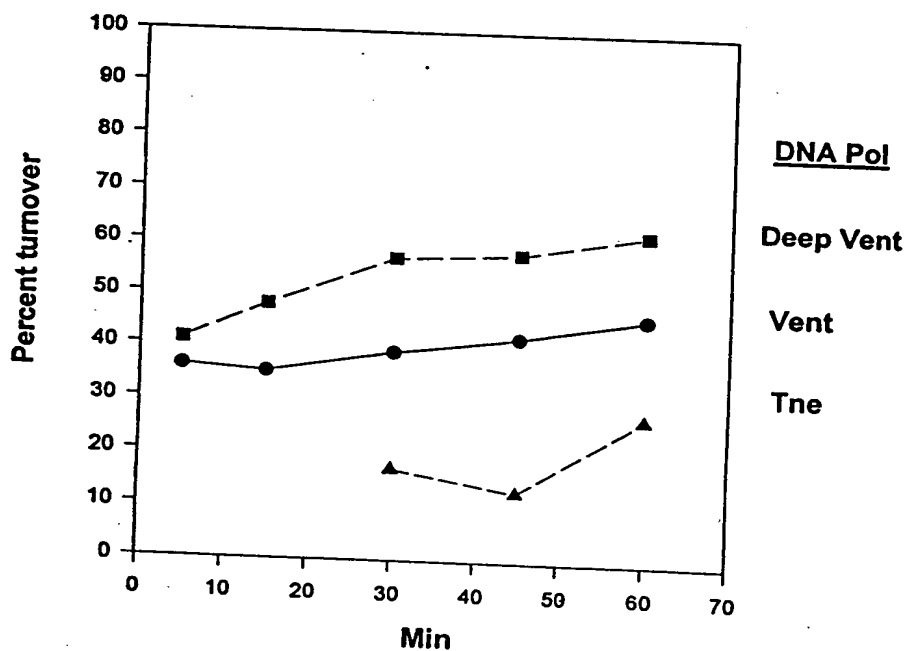
got Tnase  
 by DNA synthesis  
 1. label

Vent DNA Pol in Vent Buffer



DNA synthesis  
and turnover  
to dNMP

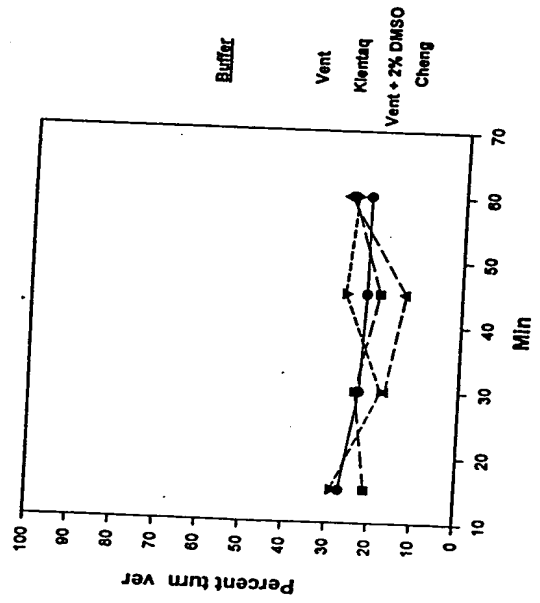
Activity in Vent Buffer



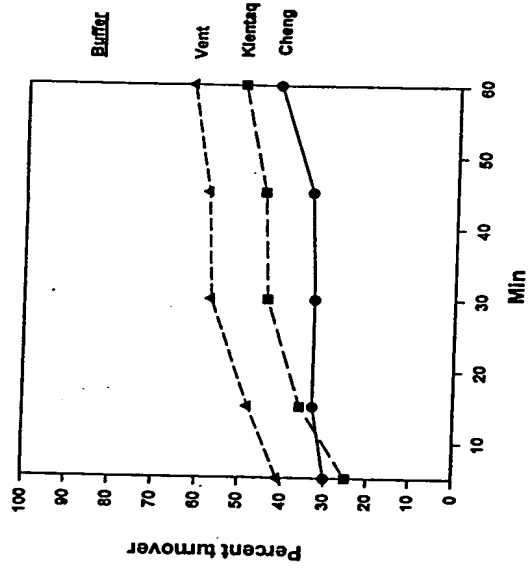
Percent turnover =  
turnover  
incorporation + turnover

Deep Vent has  
higher turnover  
than Vent as  
expected. Tne  
is ~2x lower  
than Vent and  
Deep Vent

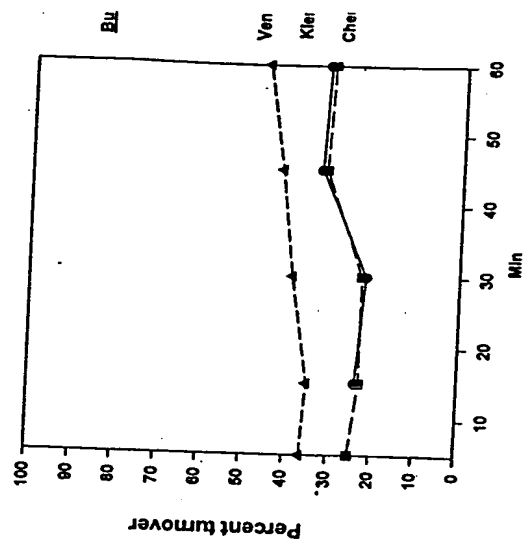
The DNA Polymerase



Deep Vent DNA Polymerase



Vent DNA Polymerase



effect of buffer on turnover is not large compared to effect on primer degradation

ed & Understood by m ,  
 aca Polcup

Date  
 11/29/94

Invented by  
 Recorded by

Date  
 11-5-94

Turnover for Vent, deep Vent  
(follow P. 61, 7)

From Page No. \_\_\_\_\_

|                               |             |           |      |
|-------------------------------|-------------|-----------|------|
| H <sub>2</sub> O              | (A)         | (B)       | (C)  |
| 5x Cheung buffer              | 399         | 487       | 489  |
| 10x KlenTaq                   | 133         | 467       | 4    |
| 10x Vent buffer               |             | 66.7      |      |
| Taq storage buffer            | 6.71        |           | 66.7 |
| 3.7 mg/ml activated DNA       | 90          |           |      |
| 1.4, 1.6, 1.8, 1.9, 10mM each | 3.33        |           |      |
| 32P dATP 10mCi/ml             | 1.21        |           |      |
| Mg(OAc) <sub>2</sub> 50 mM    | 1.6 $\mu$ l |           |      |
| MgSO <sub>4</sub> 100 mM      |             | 8 $\mu$ l |      |
| DM50 100 $\mu$ l              |             |           |      |

|                        |         |     |     |       |     |     |                 |     |
|------------------------|---------|-----|-----|-------|-----|-----|-----------------|-----|
|                        | 0.65 ml |     |     | 0.633 |     |     | 2.633.65 use 1. |     |
|                        | (1)     | (2) | (3) | (4)   | (5) | (6) | (7)             | (8) |
| Taq storage buffer     | 195     | 195 | 195 | 190   | 190 | 190 | 190             | 190 |
| Vent 0.08 $\mu$ l      | 4       |     |     | 4     | 4   | -   | 4               |     |
| Deep Vent 0.08 $\mu$ l |         | 4   |     |       | 4   |     |                 | 4   |
| Taq 0.07 $\mu$ l       |         |     | 4   |       |     | 4   |                 |     |
| H <sub>2</sub> O       |         |     |     |       |     |     |                 |     |

primers to 70°C, start by addition of pol 5 6

remove 15  $\mu$ l to 5  $\mu$ l 0.2 M EDTA → spot 15  $\mu$ l on 6  
and remove 5  $\mu$ l to 5  $\mu$ l Kill solution (20  $\mu$ mol/ml dATP  
100 mM EDTA) at 90°C

0 5 15 30 45 60 min  
spot 2  $\mu$ l on PEI resolve in 1M LiCl

\* dilutions of pols  
same as P. 81

Results: see graph on P. 81

Witnessed &amp; Understood by me,

Deena a Bolour

Date

11/29/94

Invented by

R cord d by

Dat

11-9-94

To Page 1

19 N

(1)

14.4

✓

✓

✓

66.7 20

✓

→ 27

✓ (0.0)

1. ul / 100 ul PCR ⇒ Cf = 0.005% Tween 20/NP40

So this makes up for no TPE here - its present in Joe's long PCR Run.

→ 1

✓

(Cp = 50 μm each)

→ 0.36

✓

(220 x 10<sup>6</sup> total cpm)

✓

(1.2 mM Mg(OAc)<sub>2</sub>)

✓

(1.2 mM Mg SO<sub>4</sub> in Klenow buffer)

4. ul

Cf = (2% DMSO)

(2 mM Mg SO<sub>4</sub> in 1X Vent buffer)

(10)

19.4

✓

(0.4 units total of each pol)

4

1

To Page N \_\_\_\_\_

Designed &amp; Understood by me,

Zachary Pokany

Date

11/29/94

Invented by

Recorded by

Date

11-9-94



g N \_\_\_\_\_

JAMP BK60%

1. Cherry mix = 564 ave
2. Klentz mix = 785
3. Vent mix = 922

Spot  
Cherry

$$75821 \text{ CPM} \left( \frac{50 \mu\text{L Rxn Vol}}{2 \times \text{spotted}} \right) \left( \frac{200}{195} \right) \left( \frac{1}{2500 \mu\text{m}} \right) \left( \frac{1}{4} \right) = 194 \frac{\text{CPM}}{\mu\text{mol}}$$

Klentz

$$(267 \frac{\text{CPM}}{\mu\text{mol}})$$

Vent

$$(314 \frac{\text{CPM}}{\mu\text{mol}})$$

pmol incorp =  
(200  $\mu\text{L}$  Rxn)

$$\frac{\text{CPM}}{\text{CPM}/\text{pmol}} \left( \frac{200}{15} \right) \left( \frac{20}{15} \right)$$

pmol turnover =  
200  $\mu\text{L}$  Rxn

$$\frac{\text{CPM} - \text{BK60}}{\text{CPM}/\text{pmol}} \left( \frac{200}{5} \right) \left( \frac{10}{2} \right)$$

$$\% \text{ turnover} = \frac{\text{pmol turnover}}{\text{pmol turnover} + \text{pmol incorp}}$$

21 75821.00  
22 104512.00

To Page No. \_\_\_\_\_

& Understood by me,

Eric Polay

Date

11/29/94

Inv nted by

R corded by

Date

11-10-94

**PAGES 88-89 OF NOTEBOOK WERE BLANK**

AT 90 out of Frederick. Project No. \_\_\_\_\_  
Book No. \_\_\_\_\_

Repeat unit assay QC for v120  
lot # EKBT1 done on P 61 spec

From Page N \_\_\_\_\_

Amplitag lot # 9957 for control  
lot EKBT1 is ~ 401 u/ml based on P.61

## 1. starting dilutions of EKBT1:

1:80 (estimate cf=5%)

1:160 (estimate cf=2.5)

lot EKBT1 5 µl

5 µl

Tag storage buffer 385 µl

795 µl

actual is 4.03 u/l

actual is 2.01 u/l

Vf = 400 µl

Vf = 800 µl

## 2. 1/600 dilutions

| serial dilution #    | 1-6 | 7-12 | 13-18 | 19-24 | 25-30 | 31-36 | 37-42 | 43-48 | 49-54 |
|----------------------|-----|------|-------|-------|-------|-------|-------|-------|-------|
|                      | I   | II   | III   | IV    | V     | VI    | A-1   | A-2   | A-3   |
| 1:80 dil             | 31  | 3    | 3     |       |       |       |       |       |       |
| 1:160 dil            |     |      |       | 3     | 3     | 3     |       |       |       |
| Amplitag 5%<br>lot # |     |      |       |       |       |       | 3     | 3     | 3     |

dilution buffer 1797 µl  
Vf = 2000 / 1800 µl

Vortex 5A  
use from 16  
20 and 40 ml

dilute I - A-3 as shown for I below:

## 3. Serial dilutions

| serial dilutions # | dilution buffer |          |
|--------------------|-----------------|----------|
| 1                  | 100 µl          | → 300 µl |
| 2                  | 100 µl          | → 300 µl |
| 3                  | 100 µl          | → 300 µl |
| 4                  | 100 µl          | → 300 µl |
| 5                  | 100 µl          | → 300 µl |
| 6                  | 1 ml of I       | → 300 µl |

dilute I - III and assay  
then dilute IV - VI and assay  
then dilute A-1 - A-3 and assay

SA I-III = 45 µl assay mix + 5 µl dil buffer, do same for IV-VI  
spot 4x 5 µl on 6 FC in one aqueous

Blank is 45 µl assay mix + 5 µl dil buffer → spot on 6 FC along with other

With ssed & Understood by me,

*Deereena Pokar*

Date

1/6/95

Invented by

Record d by

Date

11-15-94

To Page N

ag N . \_\_\_\_\_

# 55-57 = Blank for I-III, IV-VI and A1-A3 respectively

58-61 = SA for I-III

62-65 = SA for IV-VI

Result:

using amphotag lot #9957 here gives a  
 unit value of ~~323.4 u/ml~~ 323.4 u/ml  
 compared to 401 u/ml (found on P.61, 10-1-94)

To Page No. \_\_\_\_\_

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Date

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Date

Recorded by

Sandra Pokany

1/6/95

10-15-94

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

New determination of EKBT1 to  
10<sup>u</sup>/μl for Larry Morley

From Page No. \_\_\_\_\_

\* will use old unit value of 401 u/μl no can use  
old dilution & Jerry used in October  
(see P 91 where final unit determination for EKBT1  
is 323.4 units/μl)

calibrated P20 (P20  
its capacity 10 μg for  
for P1000 (P1000) use  
37 μl which gives  
391 μg

Tag storage buffer 391 μl

Tag lot # EKBT1  
("401" u/μl)

10 μl

\* see above

$V_p = 401 \mu\text{l}$  (10  $\frac{\text{units}}{\mu\text{l}}$ )

1. Bring Tag storage buffer to room Temp.
2. Bring small aliquot aliquot of EKBT1 (main stock)  
to room temp.
3. deliver 10 μl Tag into 391 μl storage buffer, rinse  
~10 times (i.e. triturate)
4. mix with P1000 to get in all storage buffer
5. vortex 5 sec
6. mix end over end in cold room 2 hr

Witnessed &amp; Understood by m ,

DeeAnna Polansky

Date

1/6/95

Invented by

R. P. R.

Recorded by

Date

11-30-94

T Page No

Tag No. \_\_\_\_\_

Equilibrate 2mL Q650 m w/ Q buffer A - 6/17  
dilute load of 3

Load @ .5 mL/min - ~~same~~ Sensitivity - .05

Wash to base w/ Buffer A - collect F.T. - @ 1 mL/min

Program - ① 5mL wash w/ Q Buffer A @ .5 mL/min  
② 20mL linear gradient 0 → 100% Q Buffer B  
@ .5 mL/min  
③ 45mL wash w/ Q Buffer B @ .5 mL/min  
collect 500  $\mu$ L fractions -

### Assay -

10  $\mu$ L of premix aliquotted to pre-labeled ependarfs -  
incubate @ 74°C for 5 min quench w/  
10  $\mu$ L of .5M EDTA - spot 3 20  $\mu$ L on 6 FIC  
filters - TCA wash

1x 10% TCA + 1% PI @ 5'

3x 5% TCA + @ 5'

2x EtOH

dry + count in LSF - Econofluor

Pool 24-35 dialyze o/N (over weekend) - against  
tag storage buffers (No detergents) -

7 - Remove ~ 1.8mL from dialysis - store in 2mL ependarfs  
HOT PINK - -20°C

To Page No. \_\_\_\_\_

Issued &amp; Understood by me,

Date

Invented by

Date

Way Fargo

6/20/95

Recorded by

S. H. m

6/19/95

Q650 - TBSO kit - 2mL column

Project No. \_\_\_\_\_

Box No. \_\_\_\_\_

147

Tag N. \_\_\_\_\_

06/16

\* FY-1

Wash + column w/ .5N NaOH -

Wash extensively w/ H<sub>2</sub>O

equilibrate w/ Q650-Buffer A - p. 146 -

Load ~ 3.5mL of Heparin pool of FY-1 @ .5mL/min

Wash with QBuffer A until baseline is reached -

Gradient - 20mL linear gradient 0-100% Qbuffer B  
@ .5mL/min collect .5mL fractions

Wash w/ 10mL of 100% Qbuffer B - collect  
.5mL fraction fractions -

Fraction collector - started then stopped after  
fraction 10/11 - Did not realize until  
gradient was finished - lost entire elution  
~~elution~~ to waste! Could have tried to  
save however I believe I washed the port  
with .2N NaOH + in the same waste container.

Fraction collector stopped b/c outside of rack was "dirty"  
and was slippery - Must be sure outside plastic is  
clean!

Can to proceed with 3'-5' exo mutant - Flush column  
with 3M KCl - Wash w/ H<sub>2</sub>O equilibrate w/  
Q650 Buffer A p. 146.

To Page No. \_\_\_\_\_

Read & Understood by me,

Date

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Date

May Longo

9/20/95

Recorded by

06/14/95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE Q650M - 3'5' x 0 minus - T

From Page No. \_\_\_\_\_

0-800mm K1 in ① pH 7.2

0806.N50/93109/8.93

| SAM | CFM1       |
|-----|------------|
| 1   | 20 188.00  |
| 2   | 22 138.00  |
| 3   | 24 180.00  |
| 4   | 26 874.00  |
| 5   | 28 830.00  |
| 6   | 30 748.00  |
| 7   | 32 1174.00 |
| 8   | 34 912.00  |
| 9   | 36 556.00  |
| 10  | 38 590.00  |
| 11  | 40 340.00  |
| 12  | 42 326.00  |
| 13  | 44 370.00  |
| 14  | 46 266.00  |
| 15  | 48 298.00  |
| 16  | 50 186.00  |
| 17  | 9928.00    |
| 18  | 198.00     |

Fracta

Pool

Pool 26-35

6/20/95

Pool 26-3  
dialyze of  
in JFAQ  
storage

6/16/95

Z-A

iotechnology

Code No. 18-1001-44

With ss d &amp; Understood by me,

Man Jones

Date

6/20/95

Invented by

R c rd d by

Date

6/12/95

To Page N

Page No. \_\_\_\_\_

purpose: continuation of pg 124 - 125

amplified linearized puc / xmr 1 using 2 different new sets of primers

36

37

38

39

tried with Tag and Tag + DV

tested Mg 1.5, 2.0, 2.5, 3.0 mM

cycling: 94° 30" 1  
(94° 30" 5) 30  
65° 1'

200 µM dNTP

.4 µM primer

product = 1275 bp.

1 U of enzyme - Tag

25 µg template

prepared 10x of each:

Tag / # 3

Tag + DV / # 3

" / # 2

" / # 2

H<sub>2</sub>O 338

330 µl

ox buffer 50

dNTP 10

1.5 2 2.5 3 mM

Mg -

7.5 10 12.5 15

primer 1 20

42.5 40 37.5 35

2 20

Template 10

50

enzyme 2

10 µl Tag + DV

4.50

45 µl / RX

added 5 µl of Mg dif. conc.

To Page N \_\_\_\_\_

ss d & Underst od by m ,

Date

Invent d by

Date

12/9/94

R corded by

12/9/94

V. Sitarman

Project No. \_\_\_\_\_

128 T. J.

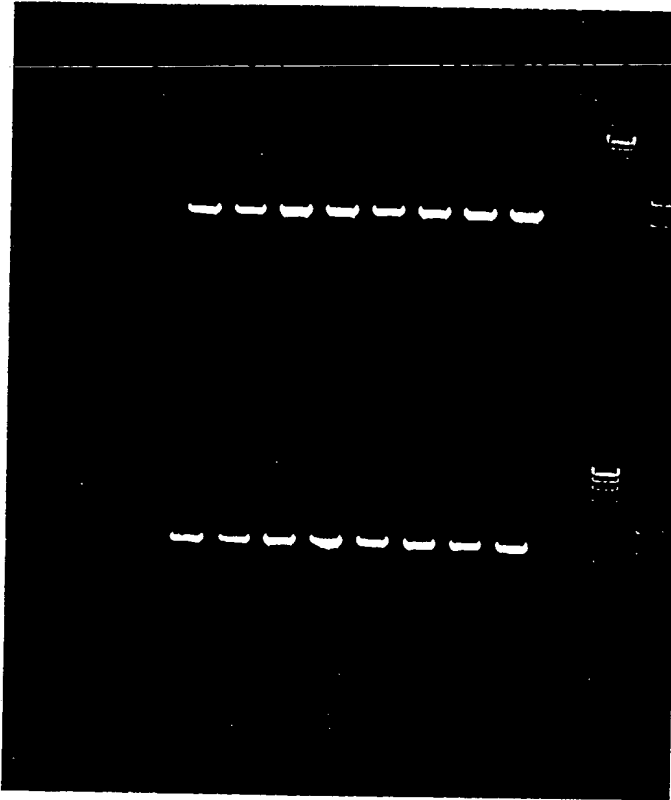
Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

Fr m Page No. \_\_\_\_\_

Tag

0 1.5 2 2.5 3 mM Mg



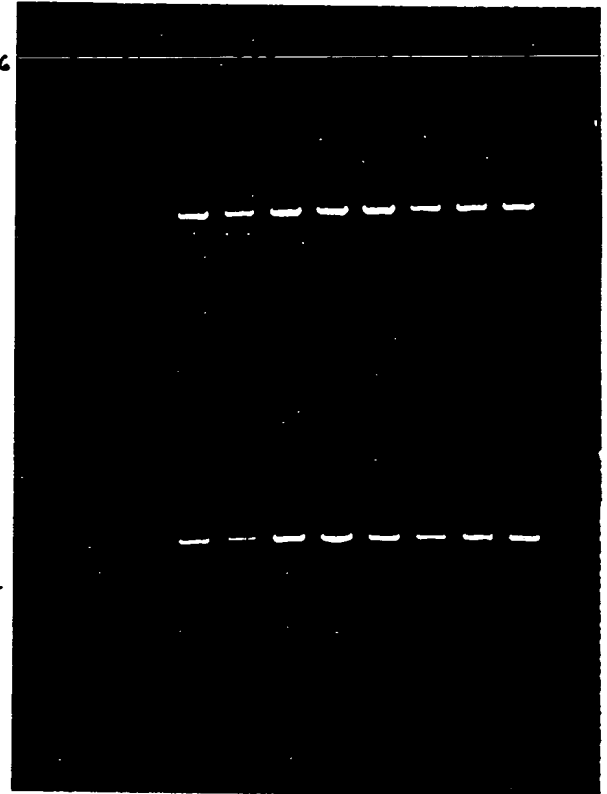
Tag + DV

0 1.5 2 2.5 3 mM

2936  
V  
37

\*3

\*2



2936 x 37

1275 bp product.

Both primers set work with Tag as well as Tag + DV

a bit of mispriming still - has to be gel purified

good range of Mg tolerance

pooled (1) Tag 1.5 mM Rx separately } with \*3  
 (2) 2.0 } set of  
 (3) Tag + DV 1.5 } primer  
 (4) 2.0 } and phenol  
 and ethanol p.p.

To Page

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Dat

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Ch. Sitarman

12/8/94

Page N. \_\_\_\_\_

loaded Rx from two tubes (duplicate of same) together is 30  $\mu$ l +  
made up the volume to 100  $\mu$ l 30  $\mu$ l

added equal amounts of phenol: chloroform: 2x amylalcohol  
removed the aqueous phase after a spin of 5' -  
phenol extracted again.

added 0.5 volume of 7M ammonium acetate and 2 vol  
of ethanol, added also a  $\mu$ l of Dextran T 500.

left at  $-20^{\circ}$ , 1.5 hr

spin down, removed ethanol, washed the pellet with  
70% ethanol, spin down, removed the sup.

spin again to remove the residual ethanol.

pellet visible, vacuum dried 5'

resuspended in 17  $\mu$ l <sup>2 TB</sup> - removed 2  $\mu$ l for gelling

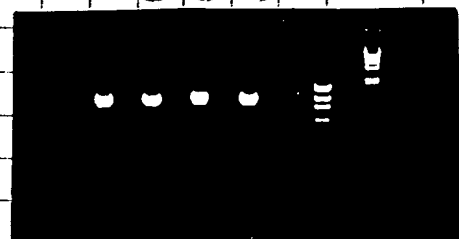
for 15  $\mu$ l added

10.5 "  $H_2O$

3.0 " 10x buffer

1.0 " Afl  $\frac{11}{11}$  (7U/x)

0.5 " Afl  $\frac{11}{11}$  (24U/x)



30  $\mu$ l incubated at  $37^{\circ}$ , 2 hr.

phenol extracted product seems to be around  $\sim 150 - 200$  ng/x2  
 $\sim 75$  ng/1x

To Page No. \_\_\_\_\_

Used &amp; Understood by m ,

Dat

12/1/84

Invent d by

Recorded by

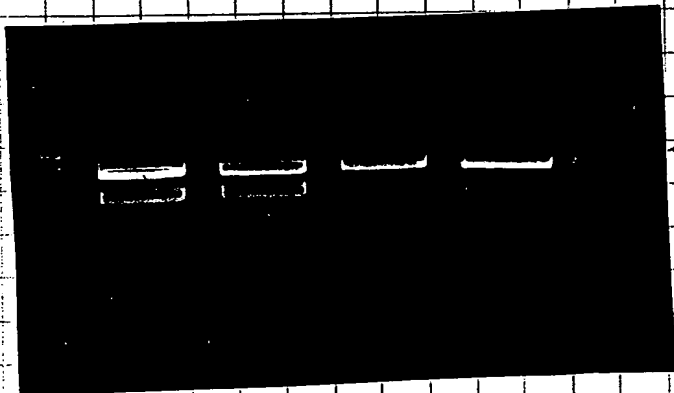
K. Sitarman

Dat

12/5/84

From Page No. \_\_\_\_\_

- 15  $\mu$ l of left over phenol chloroform extracted & ethanol pptd. insert & was cut with  $\Phi$ ak II and  $\Phi$ ak 3 in NEB buffer 4 for 2 hrs at 37°
- Run on 1% gel and transferred to DEAE paper and eluted the fragment in high salt buffer, over the 1M NaCl, 0.1M Tris pH 8.0, 5mM EDTA
- spun down the ethanol buffer, added 50  $\mu$ l more & centrifuged, poured the ethanol, ethanol added in  $\sim$  150  $\mu$ l  $\sim$  500  $\mu$ l in the presence of 1  $\mu$ l of deprotein T. 400.
- left at 70° 2 1/2 hrs, resuspended in 15  $\mu$ l after ethanol wash, in TE.



extracted to same as insert!

$$\text{loaded} \sim 75 \text{ ng} \times 15 \mu\text{l} = 1125 \text{ ng} \quad (1275 \text{ bp})$$

$$= 772 \text{ ng} \quad (875 \text{ bp})$$

$$\sim 50\% \text{ recovery} = \sim 386 \text{ ng} / 15 \mu\text{l}$$

$$= \sim 25 \text{ ng} / \lambda$$

To Page \_\_\_\_\_

Witnessed &amp; Understood by me,

Date

12/18/94

Initiated by

Date

12/12/94

Recorded by

J. Stamen

Project No. \_\_\_\_\_

28

Book No. \_\_\_\_\_ TITLE \_\_\_\_\_

From Page No. \_\_\_\_\_

3/8/95 wed

- cfg. the samples for 10-15 min.
- discarded the supernate & rinsed the pellet w/ 70% EtOH
- dried the pellet @ 55°C heat block or @ room temperature
- dissolved the DNA in 50.0 µl TE.

50M Rxn

3/8/95 wed.

Annealing Rxn.

|                            | + Primer (2899) | - Primer (2899) |
|----------------------------|-----------------|-----------------|
| H <sub>2</sub> O -         | 3.0 µl          | 4.0 µl          |
| 5x Buffer                  | 2.0 µl          | 2.0 µl          |
| pp. 1 <sup>st</sup> 55 DNA | 4.0 µl          | 4.0 µl          |
| (200mg/4x) oligo           | 1.0 µl          | —               |
| TV                         | 10.0 µl         | 10.0 µl         |

Incubated @ 70°C - 75°C for 2 min. (to eliminate non-spf. bin)  
" @ 37°C - 40°C for 2 min.

Synthesis Rxn

|   |          |
|---|----------|
| Annealing Rxn -                           | 10.0 µl. |
| 5mL 10x buffer -                          | 2.0 µl   |
| H <sub>2</sub> O -                        | 6.0 µl   |
| T <sub>4</sub> /T <sub>7</sub> DNA poly - | 1.0 µl   |
| T <sub>4</sub> DNA ligation -             | 1.0 µl   |
| TV -                                      | 20.0 µl. |

Incubated @ 37°C for 10 min.

|                 |        |
|-----------------|--------|
| Synthesis rxn - | 2.5 µl |
| TE -            | 8.0 µl |
| loading dye -   | 1.0 µl |

- ran the sample on the gel
- the picture on the next page, # 29.

To Page No

Witness d &amp; Und rsto d by m ,

Date

4/12/95

Inv nted by

R c rded by

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4/12/95

Page N \_\_\_\_\_



(con'd from pg. 28)

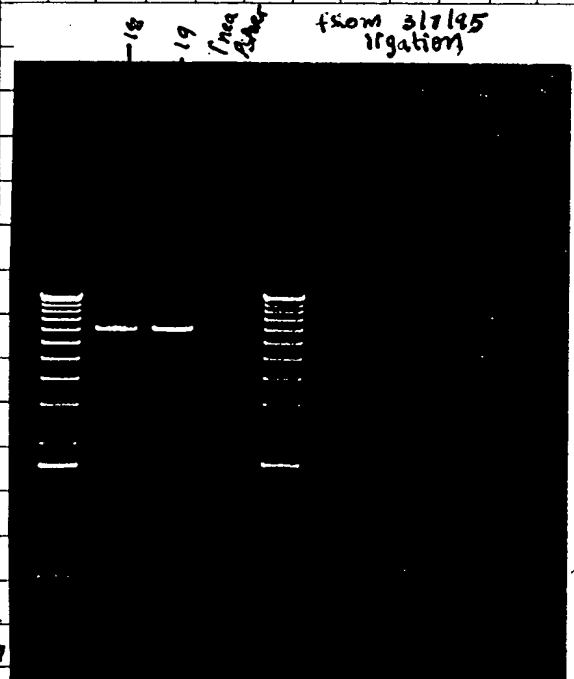
+2899 (w/ primer) oligo forms a ds DNA...

+2899 fragment looks brighter because

Et. Bromide binds to it <sup>(DNA)</sup> better. -2899Primer binds but ~~it~~ does not hold strongly

∴ the DNA fragment looks fainter or light, less Et. Bromide is able to bind.

(con'd on pg. 41)

100 bp ladder  
mp18  
mp19  
ligation  
TVLigation from 3/7/95 (pg. 26)H<sub>2</sub>O - 8.0  $\mu$ l5X buffer - 4.0  $\mu$ lmp18 - 2.0  $\mu$ linsert - 4.0  $\mu$ lligation - 2.0  $\mu$ lTV - 20.0  $\mu$ lH<sub>2</sub>O - 8.0  $\mu$ l5X buffer - 4.0  $\mu$ l(vector) mp19 - 2.0  $\mu$ linsert - 4.0  $\mu$ lligation - 2.0  $\mu$ lTV - 20.0  $\mu$ l

- Incubated both samples for 1 hour @ room temp.

|                               |                   |
|-------------------------------|-------------------|
| 100.0 $\mu$ l competent cells | } xfection cells. |
| 3.0 $\mu$ l DNA               |                   |

xfection

10% mp18 / mp19

90% mp18 / mp19

Control

ran mp18 on 3/10  
(used DNA from  
3/10/95 again on  
3/15/95 pg. 32)

To Page No. \_\_\_\_\_

Designed &amp; Understood by me,

Date

Invented by

Date

Recorded by

4/12/95

4/12/95

From Page No. \_\_\_\_\_

— 10% mp 18 / mp 19

added { 4.0 mL 2x YT TOP Agar  
 100.0  $\mu$ L X-Gal 4%

5.0  $\mu$ L IPTG 200 mM (inducer = repressor gives tighter affinity)

60.0  $\mu$ L lawn cells

10.0  $\mu$ L x fraction cells. (after heat shock for 35 sec.)

— 90% mp 18 &amp; mp 19.

Same way as 10%

— Control

100.0  $\mu$ L X-Gal

5.0  $\mu$ L IPTG

60.0  $\mu$ L lawn cells.

See to 3:45 PM

T Pag N

With ssed &amp; Understood by me,



Date

4/11/95

Inv nt d by

R c rded by



Dat

4/12/95

miniprep and digest of  
pUC 19 PCR products

Project N \_\_\_\_\_  
B ok No. \_\_\_\_\_

Exhibit 18  
Appl. No. 09/558,421

93

ag No. \_\_\_\_\_

11-8-94 received plates from Kala S. for pUC19 PCR  
with Top + Deep Vent and no  $Mn^{++}$  or dNTP bias.  
picked 20 white colonies and 2 Blue for 2 ml LB+100  $\mu$ g  
overnights at 30°C.

12-9-94

miniprep as per (p 41, 4)  
using 0.5 ml cells.

resuspended DNA in 50  $\mu$ l TE + RNase

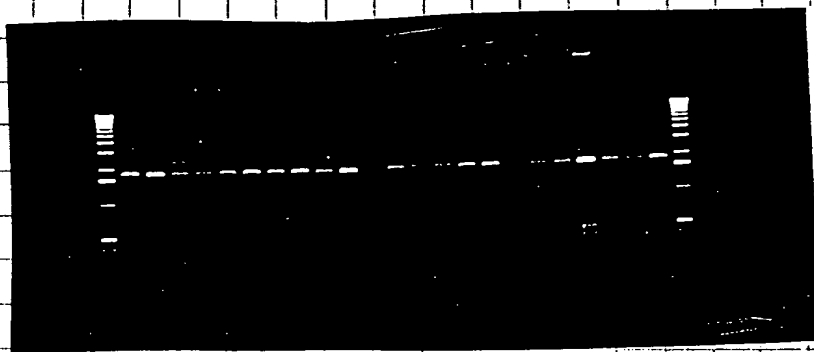
digest each (# 21, 22 <sup>are from</sup> the 2 blue colonies)  
as follows (tube 23 is 0.2 ml of 0.8  $\mu$ g/ml pUC19

|                    |            |   |   |   |      |
|--------------------|------------|---|---|---|------|
| 0                  | 11.6       | ✓ | ✓ | ✓ | 569  |
| buffer 4 (10x)     | 2          | ✓ | ✓ | ✓ | 94   |
| H <sub>2</sub> O   | 5 $\mu$ l  |   |   |   |      |
| RI 10 $\mu$ g/l    | 0.5        | ✓ | ✓ | ✓ | 23.5 |
| uH 10 $\mu$ g/l    | 0.5        | ✓ |   |   |      |
| + II 240 $\mu$ g/l | 0.1        | ✓ | ✓ | ✓ | 4.7  |
| I III 7 $\mu$ g/l  | 0.3        | ✓ | ✓ |   | 14.1 |
|                    | 20 $\mu$ l |   |   |   |      |

37°C, 2 hours

cocktail for 47 Rn  
use 15  $\mu$ l Rn  
5  $\mu$ l DNA

Result:  
all are  
full length  
loc 2



2 loc 2 gives 2 bands

sed & Understood by m ,

Deena Baker

Date

11/6/95

Invented by

Recorded by

To Page No. \_\_\_\_\_

Date 7-94

12-9-94

150

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE Tne - 3 ~~5~~ <sup>4</sup> ~~10~~ <sup>10</sup> minus -  
3-51

From Page No. \_\_\_\_\_

# B Enzyme titration -

Draw - 2 vials of Tag mix - add 22  $\mu$ l to the total of 2 dCTP-32P -

aliquot 48  $\mu$ l to pre-labeled eppendorf's - on ice -  
add 1, 2, 4  $\mu$ l of diluted enzyme - incubate 10' @ 74°C in a heat wrap block - quench w/ 10  $\mu$ l of 5% EDTA - Spot 30  $\mu$ l on GFLC - Wash

1X 10% TCA 1/1 Pi  
3X 5% TCA  
2X 5% TCA

dry and count in LSC  
under heat lamp.

| SAM | CPM1      |
|-----|-----------|
| 1   | 1864.00   |
| 2   | 2938.00   |
| 3   | 2940.00   |
| 4   | 940.00    |
| 5   | 1658.00   |
| 6   | 2606.00   |
| 7   | 404.00    |
| 8   | 320.00    |
| 9   | 732.00    |
| 10  | 152.00    |
| 11  | 306.00    |
| 12  | 384.00    |
| 13  | 126.00    |
| 14  | 238.00    |
| 15  | 326.00    |
| 16  | 118.00    |
| 17  | 106.00    |
| 18  | 134.00    |
| 19  | 112220.00 |

OK  
6/29/95

To Page N

Witnessed & Understood by me,

Date

Invented by

Date

Man Longo

6/29/95

Recorded by

6/27/95

ge N \_\_\_\_\_

6/27

| IM | CPM1     |
|----|----------|
| 1  | 6084.00  |
| 2  | 10302.00 |
| 3  | 8286.00  |
| 4  | 3506.00  |
| 5  | 4842.00  |
| 6  | 5272.00  |
| 7  | 1370.00  |
| 8  | 1842.00  |
| 9  | 3392.00  |
| 10 | 182.00   |
| 11 | 85826.00 |
| 12 | 92658.00 |
| 13 | 92494.00 |

.043

7.20 .072

.050

8.5

5.7

5.27

56/12/95

nl - Pool - .075 u/w -> ASD Units total -

TIME AVG H#

|      |      |
|------|------|
| 0.50 | 35.0 |
| 0.50 | 36.0 |
| 0.50 | 40.0 |
| 0.50 | 48.0 |
| 0.50 | 38.0 |
| 0.50 | 42.0 |
| 0.50 | 52.0 |
| 0.50 | 40.0 |
| 0.50 | 40.0 |
| 0.50 | 43.0 |
| 0.50 | 42.0 |
| 0.50 | 44.0 |
| 0.50 | 49.0 |
| 0.50 | 44.0 |
| 0.50 | 42.0 |
| 0.50 | 54.0 |
| 0.50 | 53.0 |
| 0.50 | 46.0 |
| 0.50 | 34.0 |

.08 u/w

ERR

.08 u/w

.063

.08

.071

.055

.075 u/w

56/12/95

SA = 70.1 cpm/pmol  
7.01 x 10<sup>5</sup> cpm/pmol

mf 6/29/95

To Page No. \_\_\_\_\_

Read & Understood by me,

Date

Invent d by

Date

Mary Lopez

6/29/95

Recorded by

*[Signature]*

6/27

purpose: To ligate the purified vector + insert and transform with appropriate controls.

ligation Rx: Tested

|                  |             |   |      |                |   |               |
|------------------|-------------|---|------|----------------|---|---------------|
| Vector           | ~ 100 ng/μl | = | 1 μl | 1 : 1 : 1      | 1 | Vector alone  |
| Insert           | ~ 25 ng/μl  | = | 4 μl |                | 2 | " + real c/po |
| 5x buffer        |             | = | 4    |                | 3 | any purified  |
| Ligase Tx        |             |   | 1    |                | 4 | Tag 1.5       |
| H <sub>2</sub> O |             |   | 10   |                | 5 | " 2           |
| 20 μl            |             |   |      | at 25°, 3 hrs. | 6 | " " 2         |
|                  |             |   |      |                | 5 | " + D.V. 1.5  |

transformation using DH5α Max eff. cells.

total produced used 2.5 μl of ligation Mix / transform 50 μl cells

initial volume after adding SOC ~ 500 μl

aliquot 25, 50 & 100 μl of each

controls diluted to 1:10 and plated 25, 50 & 100 μl. normal transformation efficiency

Vector only - few blues because of the contamination 1.5 x 10<sup>9</sup> no whites

Vector + insert - ligated w/o any purification, lots + lots of colonies transformation quite efficient.

Tag + D.V. } 2 mM Mg - didn't work

Tag alone } 1.5 mM Mg

Tag " } Very few colonies in 25 μl + 50 μl  
Tag + D.V. } no μl slightly better. No deep blue for better than Tag alone, however, are so low to make a call

- all purified gave low efficiency of transformation compared to un " - Vector + insert

Page No. \_\_\_\_\_

3/14/95 TUE

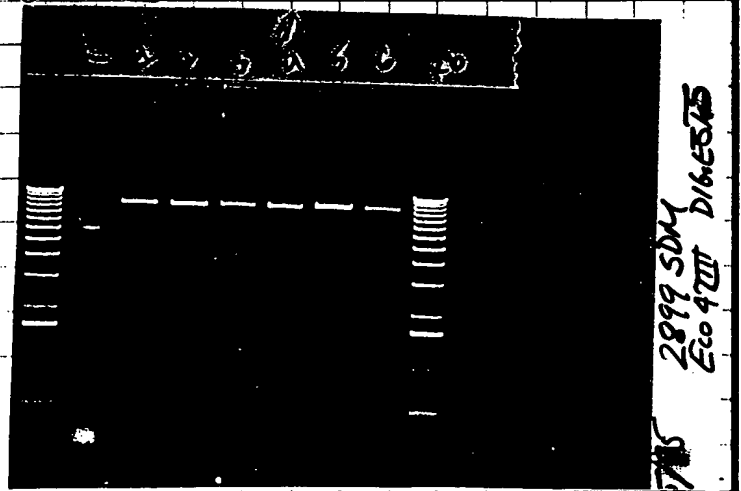
~~Reese~~: miniprep

1.0 ml culture of T-nealmp19 grown for 5 hours @ 37°C in  
6 different glass tubes  
transferred 1 ml cell to the 6 different labelled eppendorf tubes.  
cfg all 6 tubes for 2 min @ room temp.  
removed supernate & saved in different tubes  
added 100  $\mu$ l S1 mixed well  
added 200  $\mu$ l S2. put the tubes on ice (mixed by inverting)  
added 150  $\mu$ l 7.5 M  $\text{NH}_4\text{OAc}$   
incubated on ice for 5 min.  
cfg for 7 min. @ room temp (4°C) NOTE: cfg in 4°C room was taken away for repair  $\therefore$  used @ RT.  
transferred supernate (400.0  $\mu$ l) to the new 6 labelled tubes  
added 800  $\mu$ l of EtOH to the 400  $\mu$ l of supernate (mixed well)  
incubated @ -70°C for 30 min.  
cfg for 2 min. @ room temp (discarded supernate)  
rinsed w/ 70% EtOH (removed supernate)  
added 50.0  $\mu$ l TE to the pellet

|                                    |     |                |
|------------------------------------|-----|----------------|
| $\text{H}_2\text{O}$ - 7.0 $\mu$ l | x 6 | = 42.0 $\mu$ l |
| buffer - 2.0 $\mu$ l               | x 6 | = 12.0 $\mu$ l |
| Eco4III - 1.0 $\mu$ l              | x 6 | = 6.0 $\mu$ l  |
| TV                                 |     | 60.0 $\mu$ l   |

added 10.0  $\mu$ l DNA<sup>+</sup> to each 6 tubes.

the map is on next page # 32. Fragments  
on all 6 tubes are ~~in~~ still present,  
y haven't gone into the mutant.  
I tried miniprep again next day.  
(started)



d &amp; Understood by me,

Date

Invented by

Date

Polansky

4/12/95

Recorded by

4/12/95

ag No. 11-8-94 received plates from Kahan S. for pUC19 PCR  
with Top + Deep Vent and no Mn<sup>++</sup> or dNTP bias.  
picked 20 white colonies and 2 Blue for 2 ml LB+100 $\mu$ <sub>A</sub>  
overnights at 30°C.

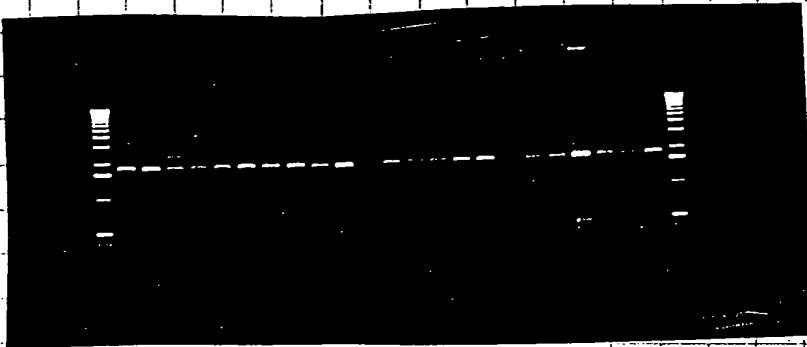
12-9-94  
miniprep as per (p 41, 4)  
using 0.5 ml cells.

resuspended DNA in 50  $\mu$ l TE + RNase

digest each (#21, 22 <sup>all from</sup> and the 2 blue colonies)  
as follows (tube 23 is 0.2 ml of 0.8  $\mu$ l pUC19  
569  
94  
23.5 } cocktail for 47 rxn  
use 15  $\mu$ l rxn  
5  $\mu$ l DNA  
4.7  
14.1  
37°C, 2 hours

|  |            |   |   |   |
|--|------------|---|---|---|
| 0                                      | 11.6       | ✓ | ✓ | ✓ |
| buffer 4 (10x)                         | 2          | ✓ | ✓ | ✓ |
| H <sub>2</sub> O                       | 5 $\mu$ l  |   |   |   |
| PL 10 <sup>u</sup> /l                  | 0.5        | ✓ | ✓ | ✓ |
| as H <sub>2</sub> O 10 <sup>u</sup> /l | 0.5        | ✓ |   |   |
| + II 240 $\mu$ /l                      | 0.1        | ✓ | ✓ | ✓ |
| I III 7 $\mu$ /l                       | 0.3        | ✓ | ✓ |   |
|  | 20 $\mu$ l |   |   |   |

Result:  
all are  
full length  
lane 2



lane 2 gives 2 bands

sed & Und rstood by m ,

Deena B. B. B.

Date

11/6/95

Invented by

R c rd dby

Date - 7-94

12-9-94

To Page No. \_\_\_\_\_

Project No. \_\_\_\_\_

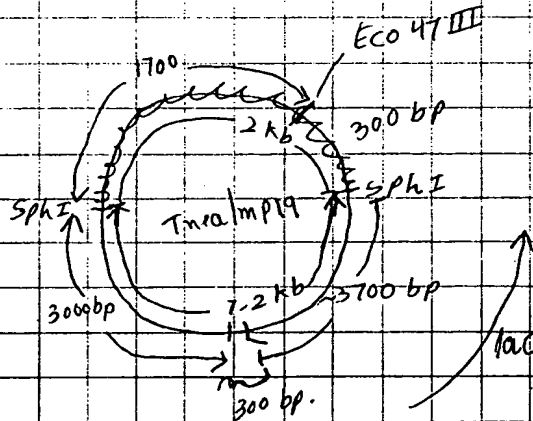
Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

32

From Page No. \_\_\_\_\_

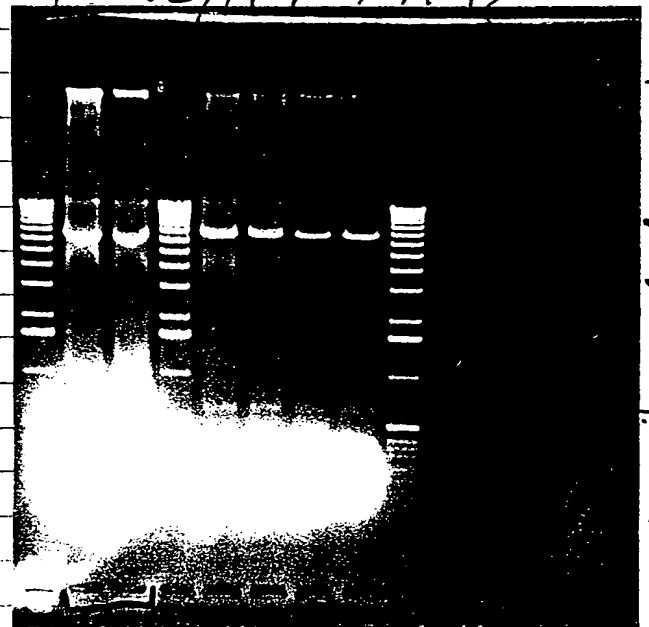
3/15/95 Wed



parent Eco 47 III  
~ 8-9 kb  
0.3 kb → (most probably won't see fragment because too small & too light)

mutant  
4 kb  
4.7 kb  
0.3 kb

DNA from date 3/10/95



H<sub>2</sub>O = 6.0 μl.  
R.Eact.6 buffer = 2.0 μl.  
mp18 DNA = 10.0 μl  
Sst I/Sph = 1.0 μl ea.  
TV 20.0 μl

H<sub>2</sub>O = 6.0 μl.  
buffer = 2.0 μl  
mp18 DNA = 10.0 μl  
Sst I/Sph = 1.0 μl ea.  
TV 20.0 μl

from pg 29  
& ran again  
on 3/15

arp  
4/12/95

3/10/95  
Sph I 700 bp

Witnessed & Understood by me,

Date

Inventor by

Date

4/12/95

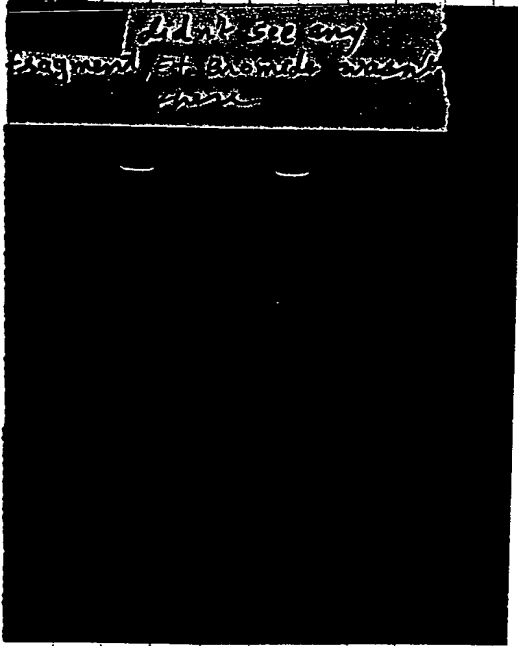
Recorded by

4/12/95

T Page

age No. \_\_\_\_\_

- Incubated both tubes @  $37^{\circ}\text{C}$  for 30 min.
- added 2.0  $\mu\text{l}$  loading dye to ea. tube
- ran both on a gel
- took picture



3/15/95 T. nea/mp

1.0 ml T. nea (sph I) / mp 19 + 2899 + sau 3AI grown for 5 hours @  $37^{\circ}\text{C}$  in 10 different glass tubes  
 after 5 hours transferred 1.0 ml culture to the 10 labelled eppendorf tubes  
 c/f all 10 eppendorf tubes @ room temperature for 2 min.  
 removed supernate & saved  
 put all 10 tubes w/ pellet & all 10 tubes w/ supernate @  $-70^{\circ}\text{C}$  overnight or until 3/16/95 Thursday.

TE: Brian had to leave @ 4:30 pm & this was a point to stop @.

To Page No. \_\_\_\_\_

ss d &amp; Understood by me,

Date

Invented by

Date

Recorded by

4/12/95

4/12/95

132

12/13/94

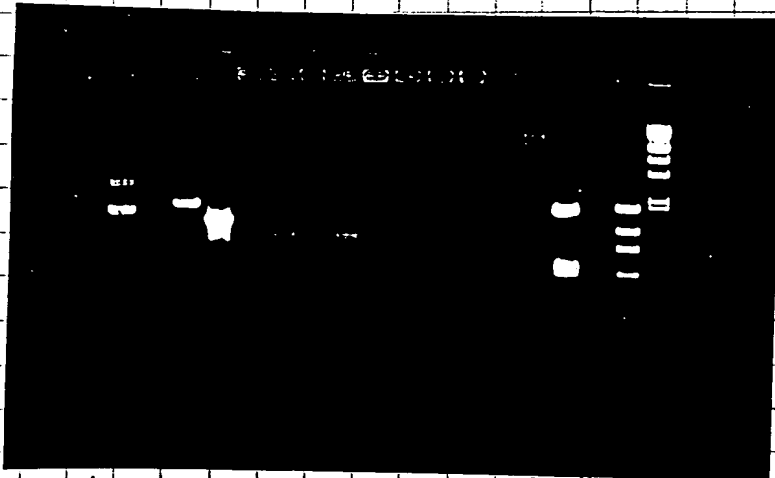
Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

From Page No. \_\_\_\_\_

- all samples, Vector, inserts, ligation Rxs run on



- ligation seems to be worked - no sign of presence of insert

- excise - linearized puc didn't show up

- purified Vector shows some contamination

- T + D.V 1.5 mM insert barely run

3 purified Vector 1 µl ~ 100 ng (still has contamination from rest of the vec)

1 insert puc ~ 0.2 µg

2 linearized puc 2.5 µg x headed 10 µl - nothing seen

4 PCR product 2 µl 12.75 bp. too little.

5 4 purified insert T + D.V 1.5 Rxs

6 4 " 2.0

7 4 " + D.V 1.5

8 4 " , D.V 2 mM

9 8 ligation 9 5 with purified Vector 3

10 9 6

11 10 7

12 11 8

13 " Vector alone

14 " 8 15

15 Vector + rest before purification

To Page

With ssed & Understood by m ,

Date

Invented by

Date

Recorded by

12/13/94

K. Sitarman

ge No. \_\_\_\_\_

since there were 8 of blues and a few whites in 100 ml of  
 1) Tag & Tag + 2 vent reactions plotted once again the  
 best of the reactions in a fresh set of plates. 1.5 ml Rx alone

|       | Blue | white | %      |
|-------|------|-------|--------|
| Tag : | 305  | 4     | 1.26 % |
|       | 92   | 1     |        |

|            | Blue | white | %      |
|------------|------|-------|--------|
| Tag + DV : | 161  | 8     | 2.48 % |
|            | 201  | 1     |        |

|             | Blue | white |
|-------------|------|-------|
| etch only : | 64   | —     |

Tag + DV is more !!

picked a few blues and

6 whites from Tag

6 " from Tag + DV

same - mini peps.

from next

page.

To Page No. \_\_\_\_\_

ed &amp; Understood by m ,

Date

Invented by

Date

Recorded by

K. Sivanathan

12/14/94

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

Received 7 clones of T41 pro gene  
from AR 12/12/94

94

From Page No. \_\_\_\_\_

Follows P115, 6

make 2 ml O/N of each in LB 100 µg/ml Amp 50 µg,  
at 30°C

inoculate ~~2 ml~~ <sup>0.4</sup> ml into <sup>40</sup> 25 ml circle grower  
with Amp, Tet (as above)  
at 30°C, shaking

started at 8 AM

Ass

11:30 AM

0.296

12:05

0.43

12:30

0.58

induce at 42°C 2 hr starting at 12:45  
found OD = 0.87 at 3 PM (store cells pellet)

(mini-preps of 1 ml from O/N)

|    |      |
|----|------|
| #1 | 1084 |
| 2  | 152  |
| 3  | 106  |
| 4  | 202  |
| 5  | 151  |
| 6  | 107H |
| 7  | 109  |

see P 157-

will try induction at

42°C, 15' and

37°C 40 min

digest with Afl III, Aat II  
and Eco RI in NEB buffer 4  
(see PG3) 2 hr, 37°C

Witnessed & Understood by me,

Domena Bolero

Date

11/1/94

Invented by

Recorded by

Date

12/12/94

To Page

**Project No.**\_\_\_\_\_

**Book No.**

**TITLE**

134

12/13/94

From Page No.\_\_\_\_

Purpose: To make more ligatin reactions at a different ratio of vector insert + to transform.

|                  |     |           |
|------------------|-----|-----------|
| Vector           | 0.5 | } n-1 ; 3 |
| insert           | 6.0 |           |
| 5x buffer        | 4.0 |           |
| T4 ligase        | 1.0 |           |
| H <sub>2</sub> O | 8.5 |           |

same the same for all Res

- Vector alone

~ Tong

Tag + Sleep Vent }

20  $\mu$ l at 25°, 3 hrs.

used 2.5  $\mu$ l of each reaction to transform DH5 $\alpha$  n efficiency cells.

included untreated pvc (monomer) Control

usual protocol.

lyotain des

Vector only : receipt - 51

Tag

|            |   |     |
|------------|---|-----|
| 25 $\mu$ l | - | 150 |
|------------|---|-----|

|    |  |  |   |     |
|----|--|--|---|-----|
| 50 |  |  | - | 352 |
|----|--|--|---|-----|

|     |  |   |     |
|-----|--|---|-----|
| 100 |  | - | 504 |
|-----|--|---|-----|

|     |   |
|-----|---|
| 1.8 | 2 |
|-----|---|

1006

| Tag | DV  |
|-----|-----|
| 1   | 1   |
| 2   | 2   |
| 3   | 3   |
| 4   | 4   |
| 5   | 5   |
| 6   | 6   |
| 7   | 7   |
| 8   | 8   |
| 9   | 9   |
| 10  | 10  |
| 11  | 11  |
| 12  | 12  |
| 13  | 13  |
| 14  | 14  |
| 15  | 15  |
| 16  | 16  |
| 17  | 17  |
| 18  | 18  |
| 19  | 19  |
| 20  | 20  |
| 21  | 21  |
| 22  | 22  |
| 23  | 23  |
| 24  | 24  |
| 25  | 25  |
| 26  | 26  |
| 27  | 27  |
| 28  | 28  |
| 29  | 29  |
| 30  | 30  |
| 31  | 31  |
| 32  | 32  |
| 33  | 33  |
| 34  | 34  |
| 35  | 35  |
| 36  | 36  |
| 37  | 37  |
| 38  | 38  |
| 39  | 39  |
| 40  | 40  |
| 41  | 41  |
| 42  | 42  |
| 43  | 43  |
| 44  | 44  |
| 45  | 45  |
| 46  | 46  |
| 47  | 47  |
| 48  | 48  |
| 49  | 49  |
| 50  | 50  |
| 51  | 51  |
| 52  | 52  |
| 53  | 53  |
| 54  | 54  |
| 55  | 55  |
| 56  | 56  |
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| 58  | 58  |
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| 60  | 60  |
| 61  | 61  |
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| 63  | 63  |
| 64  | 64  |
| 65  | 65  |
| 66  | 66  |
| 67  | 67  |
| 68  | 68  |
| 69  | 69  |
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| 71  | 71  |
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| 73  | 73  |
| 74  | 74  |
| 75  | 75  |
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| 80  | 80  |
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| 84  | 84  |
| 85  | 85  |
| 86  | 86  |
| 87  | 87  |
| 88  | 88  |
| 89  | 89  |
| 90  | 90  |
| 91  | 91  |
| 92  | 92  |
| 93  | 93  |
| 94  | 94  |
| 95  | 95  |
| 96  | 96  |
| 97  | 97  |
| 98  | 98  |
| 99  | 99  |
| 100 | 100 |

|    |  |  |     |
|----|--|--|-----|
| 25 |  |  | 124 |
|----|--|--|-----|

|    |  |  |     |
|----|--|--|-----|
| 50 |  |  | 274 |
|----|--|--|-----|

|     |     |
|-----|-----|
| 100 | 487 |
|-----|-----|

0.72

885-

In this set T+D.V. : seems to be  
still a very hard count.

1 Vector  
 2 Vector + rest  
 3 Tag  
 4 Tag + D.V.

Witnessed & Understod by m ,

**Date**

Inv nt d by

Date \_\_\_\_\_

**Recorded by**

To Pag N

12/12/94

rded by  
K. Karaman

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_ TITLE \_\_\_\_\_

34

From Page No. \_\_\_\_\_

3/16/95 Thurs.

con'd from page 33 3/15/95 wed. MINIPREP

- took the pellet out from  $-70^{\circ}\text{C}$  (10 eppendorf tubes)
- added 100  $\mu\text{L}$  S1 mixed well
- added 200  $\mu\text{L}$  S2 put all 10 tubes on ice. mixed
- added 150  $\mu\text{L}$  1.5 M  $\text{NH}_4\text{OAc}$
- incubated on ice for 5 min.
- cfg all 10 tubes for 5 min. @ room temp. ( $4^{\circ}\text{C}$ )
- transferred 400  $\mu\text{L}$  of supernate to the new 10 labelled tubes
- added 800  $\mu\text{L}$  EtOH Mixed well
- incubated all 10 tubes for 30 min. @  $-70^{\circ}\text{C}$ .
- cfg & discard for 2 min. @ room temp.
- discarded supernate & washed pellet with 70% EtOH.
- added 50  $\mu\text{L}$  TE to all 10 tubes w/ pellet

|                      | tubes             |                                |
|----------------------|-------------------|--------------------------------|
| $\text{H}_2\text{O}$ | 1.0 $\mu\text{L}$ | $\times 10 = 10.0 \mu\text{L}$ |
| buffer               | 2.0 $\mu\text{L}$ | $\times 10 = 20.0 \mu\text{L}$ |
| ECO 47III            | 1.0 $\mu\text{L}$ | $\times 10 = 10.0 \mu\text{L}$ |
|                      | T.V               | $= 100.0 \mu\text{L}$          |

- added 10.0  $\mu\text{L}$  from T.V to all other 9 tubes
- added 10.0  $\mu\text{L}$  DNA to each 10 tubes

- incubated @  $37^{\circ}\text{C}$  for 30 min.
- added 2  $\mu\text{L}$  loading dye
- ran all 10 samples on a gel for 1 hour @ 190 V
- took a picture

picture on pg 35

To Page N

Witness d &amp; Understood by me,

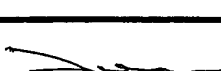


Dat

4/12/95

Invented by

Recorded by

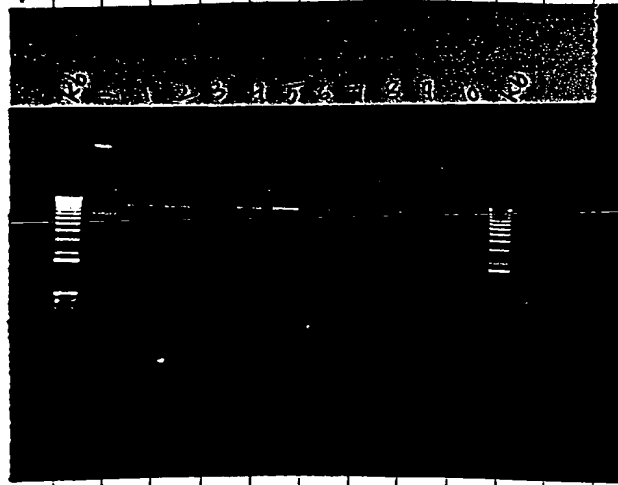


Dat

4/12/95

Ag No. \_\_\_\_\_

parent & mutant should look like  
ECO 47 III



8.9 kb  
4.5 kb  
4.4 kb  
0.3 kb

parent  
8.9 kb  
0.3 kb

mutant

4.5 kb  
4.4 kb  
0.3 kb

may probably be too light to see

NOTE: In this we could see parent & some mutant. mutant is seen on # 5, 6, 7, 8

Ad

To Page No. \_\_\_\_\_

Read & Understood by me,

*[Signature]*

Date

4/12/95

Invented by

Recorded by

*[Signature]*

Date

4/12/95

Extract TFI cells and heat  
treat as per P. 115, 6

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

Results on P 97

Exhibit 21

Appl. No. 09/558,421

15

9 N - with 1ml Tag ext buffer (P117, 3) (+ PMST and 15ME

Pol array

maxA

(for 9 x  $V_p = 10.1$  Rxn)  
(for 27 Rxns)

Red is  
3-23-91  
cocktail

x TFI Reaction  
(equivalent -  
mM Tris pH 9  
Ammonium

45  $\mu$ l

✓ ✓

131 67.5

25 mM  
NTPs 10 mM each  
H<sub>2</sub>O  
2 CDP  
3.7 mg/ $\mu$ l

72

✓ ✓

54

(50 mM)  $V_p = 2$  mM  
200  $\mu$ l rxn

18

✓ ✓

27

597

✓ ✓

870

10.5

✓ ✓

4

122

✓ ✓

183

$V_p$  855

(use 95  $\mu$ l / 100  $\mu$ l Rxn)

$V_p = 1215$

use 45  $\mu$ l / 50  $\mu$ l  
Rxn

1 2 3 4 5 6 7 8  
95  $\mu$ l → 0

202

5

5

5

5

5

5

5

0.1  $\mu$ l

0.5

4.5

100  $\mu$ l

72°C remove 20  $\mu$ l to 5  $\mu$ l 0.2 M EDTA at 15, 30, 60 min  
Results on P 97

note - all lanes have thermostable activity - eg #107 is ~0.14  $\mu$ l  
Tag is ~25  $\mu$ l (P36) and 40 mg/ $\mu$ l in Fr I (P 37)

afford on Fr I's

To Page No. \_\_\_\_\_

d & Understood by m ,

Date

Invented by

Date

evan Boking

6/95

Rec rded by

12-14-91

Page No. \_\_\_\_\_

miniprep 2 blues  
 Top 6 white  
 DV 6 "

12/14/94

Plated again the left ones from 12/13/94 transformation R<sub>0</sub>

| <u>b</u> | <u>co</u> |       |                     |       |
|----------|-----------|-------|---------------------|-------|
| 35       | -         |       |                     |       |
| 528      | 6         |       |                     |       |
| 437      | 4         | 1.1 % | average }<br>2 days | 1.4 % |
| 567      | 7         |       |                     |       |
| 1532     | 17        |       |                     |       |
| 569      | 4         |       |                     |       |
| 502      | 3         |       |                     |       |
| 579      | 5         | 0.7 % | "                   | 0.7 % |
| 1650     | 12        |       |                     |       |

ult: Once again # of whites are less than both  
 of & Tag + DV give ~ 1-2% mutants + these  
 are not satisfactory to make a call. Tag: T+DV = 2:1!  
 To Page No. \_\_\_\_\_

Read & Understood by me,

Date

Invested by

Date

Recorded by

A. Sitarasman

12/15/94

Results on TFI pol activity from P95

Project N .

Exhibit 22

Appl. No. 09/558,421

Book No.

97

| SAM  | CPM1      | pmol | u/ml         |
|------|-----------|------|--------------|
| 207  | 10297.00  | 473  | 0.037        |
| 102  | 22380.00  | 1028 |              |
|      | 42363.00  | 1946 |              |
|      | 25336.00  | 1164 | 0.09         |
| 106  | 44240.00  | 2033 |              |
|      | 82378.00  | 3786 |              |
|      | 36103.00  | 1659 | 0.129        |
| 107  | 58201.00  | 2675 |              |
|      | 90720.00  | 4169 |              |
|      | 39104.00  | 1797 | 0.144/ml     |
| 108  | 57842.00  | 2657 |              |
|      | 106183.00 | 4880 |              |
|      | 4229.00   | 194  | 0.015        |
| 109  | 8062.00   | 370  |              |
|      | 17941.00  | 824  |              |
|      | 20144.00  | 926  |              |
| 151  | 37486.00  | 1722 | 0.134        |
|      | 65420.00  | 3006 |              |
|      | 23430.00  | 1077 | 0.083        |
| 152  | 43025.00  | 1977 |              |
|      | 71820.00  | 3301 |              |
|      | 37673.00  | 1731 | 0.7 u/ml     |
| TFI  | 63089.00  | 2899 |              |
| 05 u | 99545.00  | 4575 |              |
|      | 871.00    | BKED |              |
| 22   | 109915.00 | 62   | 10 u/ml/pmol |

| 5 u | Bradford | I' (after 5' 90°) | u/mg    |
|-----|----------|-------------------|---------|
| 201 | 204      | 0.44              | 83      |
| 106 | 225      | 0.49              | 185     |
| 107 | 251      | 0.55              | 235     |
| 108 | 220      | 0.48              | 291 u/m |
| 109 | 230      | 0.51              | 29      |
| 151 | 23       | 0.51              | 265     |
| 152 | 20       | 0.44              | 190     |

note: get ~ 10,000 - 20,000 u/mg from TFI Tag (Fr I')  
 done, eg see P 104 - 105, 7 and P 36-37 (this book)

~~if ~ 5 mg/ml~~

~~still only ~~~

get ~ 200 unit/mg in I'

~~resp~~ so ~ 100x less activity here

To Page No. \_\_\_\_\_

sed & Understood by me,

Date

Inv nted by

Date

even a Polan

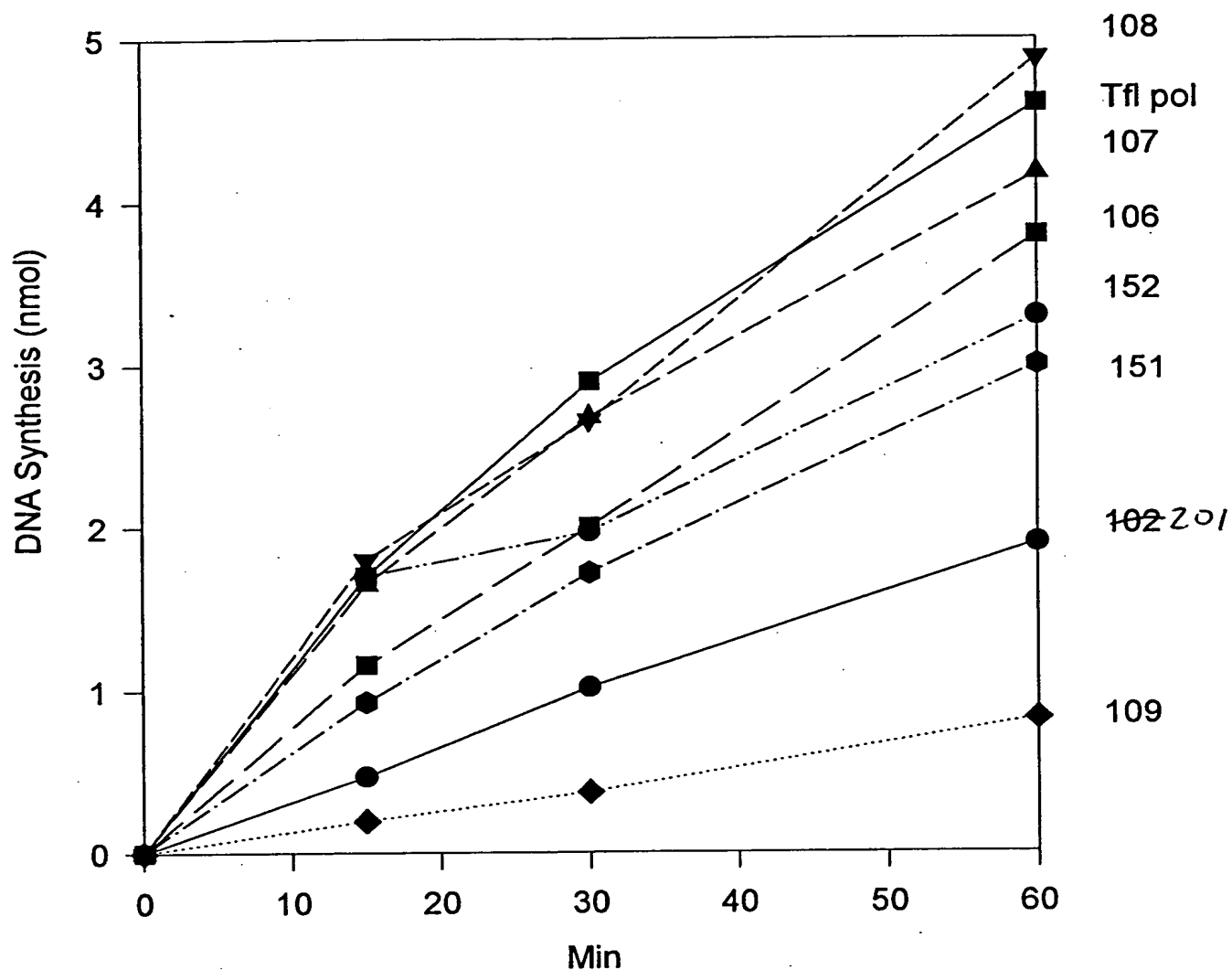
11/6/95

Recorded by

17-15-94

From Page No. \_\_\_\_

## Tfl clones



To Page I

Witnessed &amp; Understood by me,

Deena Polansky

Date

1/6/95

Invented by

D. Polansky

Recorded by

Date

12/15/94

End label 50 mer for test  
of STMP incorp opposite template U

Project N. \_\_\_\_\_

Book No. \_\_\_\_\_

99

Page No. \_\_\_\_\_ End label as per ~~P128~~ P128, 6 (and P132, 6)

ligo 733 (30mer)

69.4 pmol/l

↓ dil 1/69.4

= 1 pmol/l

5 X kinase buffer

PNK

32P ATP

H<sub>2</sub>O

1 µl

2

21

40 µl

↓ 37°C, 30' → 55°C, 5'

40 µl

15 µl

74 µl

73 µl

132 66 µl

10 pmol

100 pmol (30 10X excess of 6780)

(.076 pmol of 32P 733)

itled.seq Length:85 Tue Nov 29 10:10:41 1994

er-Lower Dimers

r positions: untitled:1U85 untitled:61L18

/Lower: the most stable 3'-dimer: 2 bp, -3.1 kcal/mol

AAAAGTCACCTGCATCAGCAATAATTGTATATTGTGGAGACCCCTGGAACCTATAGGAATTAATGAAGGAGAATTCGGGTC  
3' ATTACTTCCTCTT

/Lower: the most stable 3'-dimer: 18 bp, -32.9 kcal/mol

AAAAGTCACCTGCATCAGCAATAATTGTATATTGTGGAGACCCCTGGAACCTATAGGAATTAATGAAGGAGAATTCGGGTC  
3' ATTACTTCCTCTTAAGGC 5'

/Lower: the most stable dimer overall: 18 bp, -32.9 kcal/mol

AAAAGTCACCTGCATCAGCAATAATTGTATATTGTGGAGACCCCTGGAACCTATAGGAATTAATGAAGGAGAATTCGGGTC  
3' ATTACTTCCTCTTAAGGC 5'

2 U<sub>2</sub>

32P 733

To Page No. \_\_\_\_\_

sed & Understood by me,

Date

Invented by

Dat

Veronica Polansky

1/6/95

Recorded by

12/15/94

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

136

12/14/94

From Page No. \_\_\_\_\_

Purpose: To try *Agarose* in balance & in transformation & whether we get better &.

L 1 = Tag 1 U (200  $\mu$ M each nucleotide) 1

L 4 = Tag 4 U " " 2

Tag: H 1 = " 1 U 200  $\mu$ M dA & sub 1 mM 3

H 4 = " 4 U " " 4

L-D 1 = Tag + DV 1 (200)

L-D 4 = " 4 " "

H-D 1 = " 1 (200 + 1 x 3)

H-D 4 = " 4 " "

Supernatant of each reaction poured together, ethanol pptd after a phenol chloroform extraction.

Resuspended in 15  $\mu$ l reaction TE sub with Afl III and Aat II in NEB buffer overnight at 37°.

2  $\mu$ l of each run on gel to see the digestion is com.

Even though Ayoub said there is enough product in PCR & some of them didn't show up on the gel after all the purification steps.

Since there is not much time to gel purify the fragments whole reaction as such, was used in the ligation reaction.

2 10  $\mu$ l of it.

To Page No. \_\_\_\_\_

Witnessed & Understood by m ,

Date

12/14/94

Initiated by

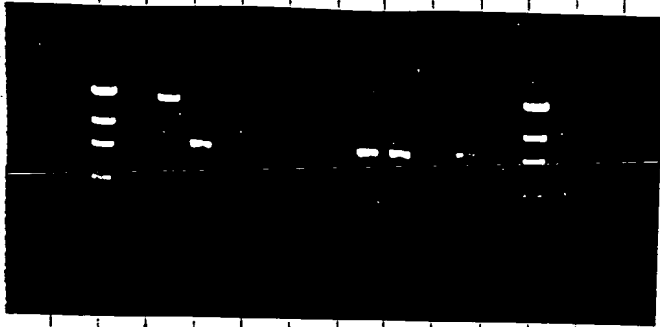
Recorded by

Dr. Shivanian

Date

12/15/94

ag N \_\_\_\_\_



Vector  
Tag  
RV  
Tag + SV

ligation

- |     |               |      |   |
|-----|---------------|------|---|
| 1.  | Vector only   |      |   |
| 2.  | Vector + rest |      |   |
| 3.  | Tag           | 2.1  |   |
| 4.  |               | 1.4  |   |
| 5.  |               | 1.1  |   |
| 6.  |               | 1.4  |   |
| 7.  | T+SV          | LD 1 | 100 µl of<br>Restriction<br>don't<br>know<br>exactly<br>how much<br>insert is there |
| 8.  |               | LD 4 |   |
| 9.  |               | HD 1 |   |
| 10. |               | HD 4 |   |

Vector 1 µl  
insert 10 µl  
ligase 1 µl  
76  
5x buffer 4 µl

200 µl at 25°, 3 hrs.

transformed all 10 (1-10 above) & control insert miniprep

To Page No. \_\_\_\_\_

Read & Understood by me,

Date

12/16/94

Inv. nted by

Recorded by

St. Blacraman

Dat

12/16/94

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_ TITLE \_\_\_\_\_

100

From Page No. \_\_\_\_\_

(1) (2) (3) (4) (5) (6) (7) (8) (9) (10)

32P 733: 678

P.S.9

10x Ultima

10x PCR buffer (Tag)

1x 20x buffer

10x Vent buffer

10x Pfu II

4 dNTPs 10mM each

Ultima 6 u/l

Tag 3 u/l EKBT (PS09)

Taq 5 u/l 11-2-34

Tfi 1 u/l

Tth (PGE) 2.5 u/l

Vent 2 u/l

Deep Vent 2 u/l

Pfu 2.5 u/l

DMSO 20 u/l

25mM MgCl<sub>2</sub>H<sub>2</sub>O

34.6 34.2 34.5 35 35 37.8 37.8 37 34.7 35 ✓

50 µl

70°C remove 10 µl to 5 µl cycle seq stop solution

at 1, 10, 20

Witnessed &amp; Understood by me,

Deena Polaris

Dat

1/6/95

Invented by

Rec rd d by

Dat

12/16/94

To Page N

ag N \_\_\_\_\_

10x ~~alt~~ <sup>alt</sup> ~~prim~~ <sup>prim</sup> 733 total / 50  $\mu$ l (7.6 nM primer total)

10 mM Tris pH 8.8, 10 mM KCl, 0.02% Tween 20

PCR buffer (BRL) cat # Y02028, 20 mM Tris pH 8.8, 50 mM KCl

(200  $\mu$ M each dNTP)

units pol (~0.125 pmol pol molecules)

$\frac{3}{1} \sim 3^{32}\text{P}733 / 1$  pol molecules

KPSA at 1X = 20 mM Tris pH 8.2, 10 mM KCl, 10 mM  $(\text{NH}_4)_2\text{SO}_4$ , 2 mM  $\text{MgSO}_4$ , 1% Triton  
 DTR = 20 mM Tris pH 7.5, 20 mM KCl, 10 mM  $(\text{NH}_4)_2\text{SO}_4$

To Page No. \_\_\_\_\_

Issued &amp; Understood by me,

Date

Invented by

Date

Seraa Polay

1/6/95

Recorded by

12/16/94

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

138 12/16/94

| From Page No. | Tag | B   | W | %   | Tag + DV | B   | W |
|---------------|-----|-----|---|-----|----------|-----|---|
| L             | 1   | 837 | 8 | 1   | LD 1     | 984 | 2 |
| L             | 4   | 777 | 7 | 1.2 | LD 4     | 599 | 7 |
| H             | 1   | 782 | 7 | 0.9 | HD 1     | 732 | 8 |
| H             | 4   | 920 | 4 | 0.4 | HD 4     | 691 | 3 |

nothing to great about!

|     |     |     |   |      |     |   |
|-----|-----|-----|---|------|-----|---|
| L 1 | 25  | 156 | 0 | LD 1 | 206 | 0 |
|     | 50  | 260 | 1 |      | 341 | 1 |
|     | 100 | 421 | 7 |      | 437 | 1 |

|     |     |     |   |      |     |   |
|-----|-----|-----|---|------|-----|---|
| L 4 | 25  |     |   | LD 4 | 101 | 2 |
|     | 50  | 51  | - |      | 208 | 2 |
|     | 100 | 119 | 3 |      | 290 | 6 |
|     | 150 | 251 | 2 |      |     |   |
|     | 200 | 356 | 4 |      |     |   |

|     |     |     |   |      |     |     |   |
|-----|-----|-----|---|------|-----|-----|---|
| H 1 | 25  |     |   | HD 1 | 25  | 95  | - |
|     | 50  | 92  | 2 |      | 50  | 216 | 2 |
|     | 100 | 119 | 2 |      | 100 | 421 | 6 |
|     | 150 | 216 | 2 |      |     |     |   |
|     | 200 | 355 | 1 |      |     |     |   |

|     |     |     |   |      |     |     |   |
|-----|-----|-----|---|------|-----|-----|---|
| H 4 | 50  | 69  | - | HD 4 | 25  | 113 | - |
|     | 100 | 128 | - |      | 50  | 248 | 1 |
|     | 150 | 315 | - |      | 100 | 330 | 2 |
|     | 200 | 408 | 4 |      |     |     |   |

To Page No.

Witnessed & Understood by me,

Date

12/16/94

Invented by

Recorded by

Dr. Sitae man

Date

12/16/94

102

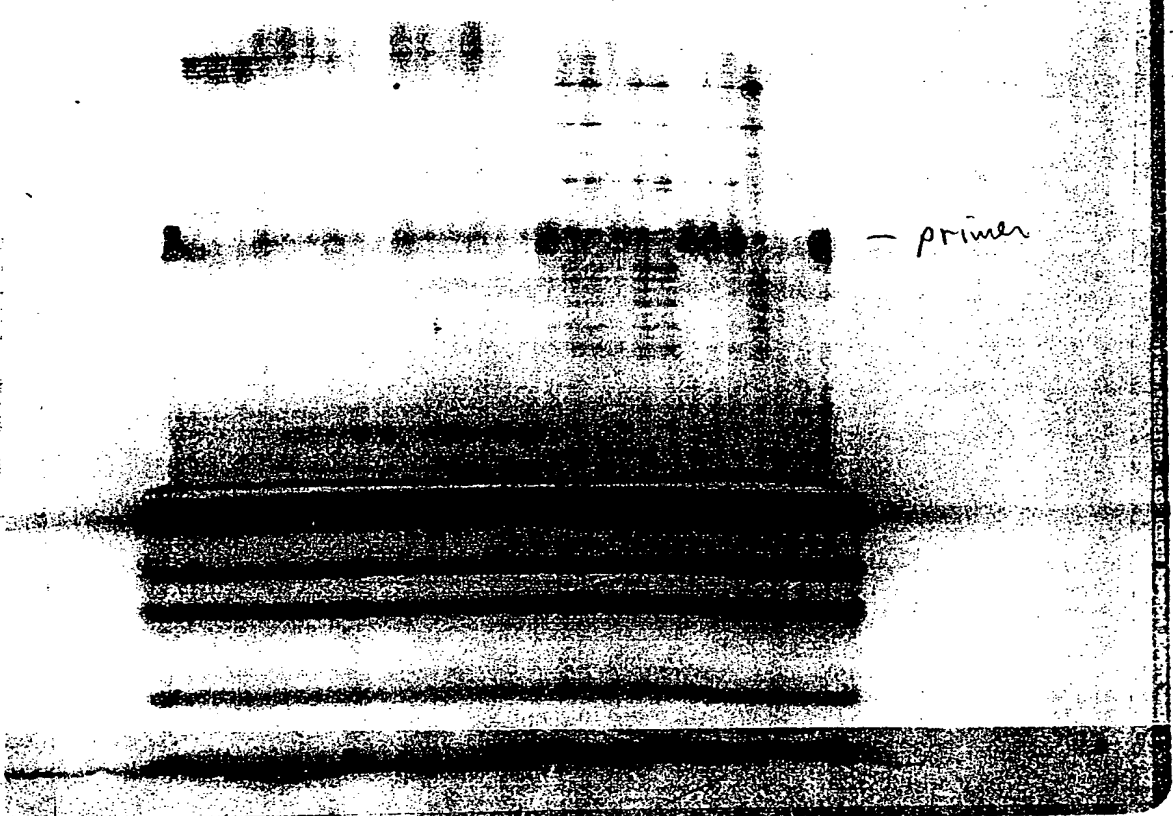
Project No. \_\_\_\_\_


Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

From Page No. \_\_\_\_\_

| Eubacteria |         |         |         |         | Archae  |           |         |         |
|------------|---------|---------|---------|---------|---------|-----------|---------|---------|
| Ultimer    | rTag    | Tne     | Tfi     | rTth    | Vent    | Deep Vent | Pfu     | D.Tok   |
| 0 1 10 20  | 1 10 20 | 1 10 20 | 1 10 20 | 1 10 12 | 1 10 20 | 1 10 20   | 1 10 20 | 1 10 20 |



<sup>32</sup>P <sup>3'</sup>  AAUAAAGACUACGUCCAC

0.11 999.87

0.50x Counts

- 12/17/94 - 09:42 pm

06.GEL

To Page 1

Witnessed & Understood by me,

*Dorinda Polansky*

Date

2/16/95

Invented by

Recorded by

Date

12/17/94

date: 1/8/95 where only (-) Mn

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

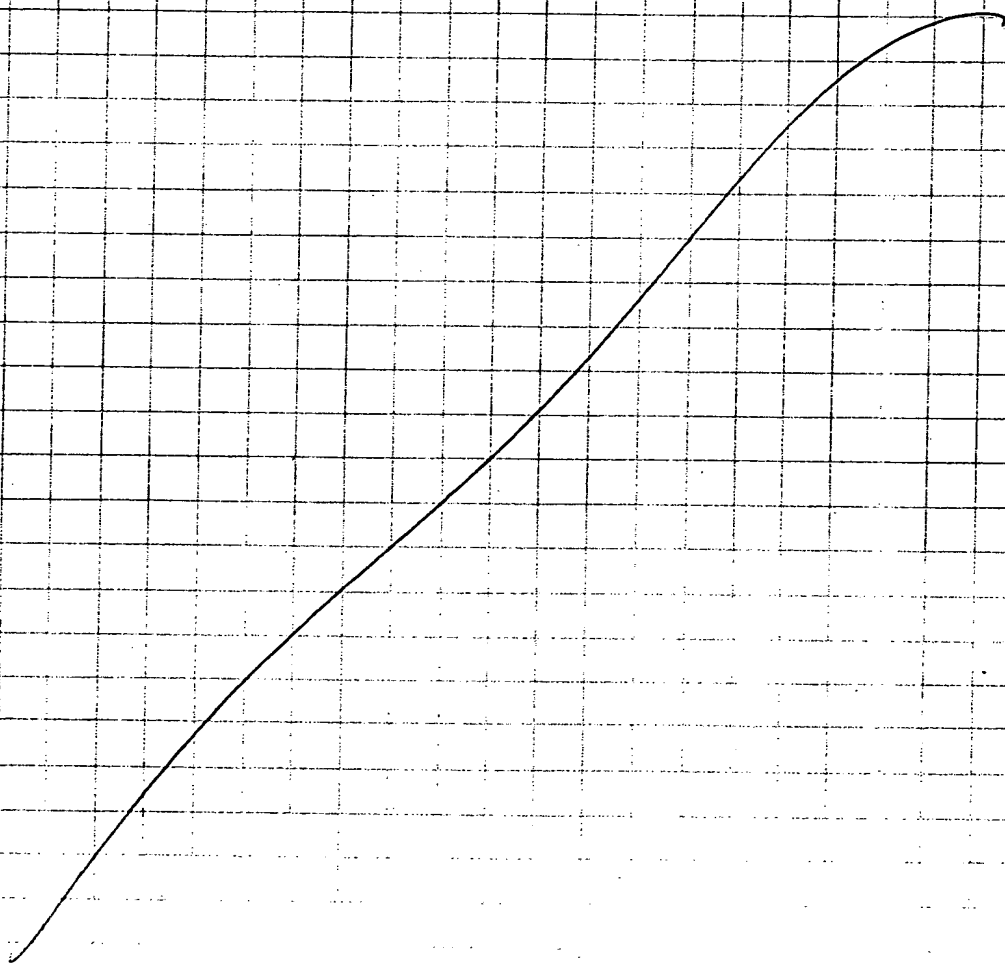
103

ag N - condition same colonies

this is to reconfirm result of P93 that dextranizing  
medium on p11C 19 give full length lac in white colonies

plates are all 0/5 = no Mn<sup>++</sup> and 5-4 Tag  
puck 5 into 2 ml LB + 100 µg 1 ml LB  
30°C 0/N

Didn't complete this experiment



Read & Understood by me,

Michaela Polak

Date

2/16/95

Invented by

Recorded by

To Page No. \_\_\_\_\_

Date

1-10-95

Picture 3 wheels from H200012. experiments  
done 1/8/95 where only (-) Mn

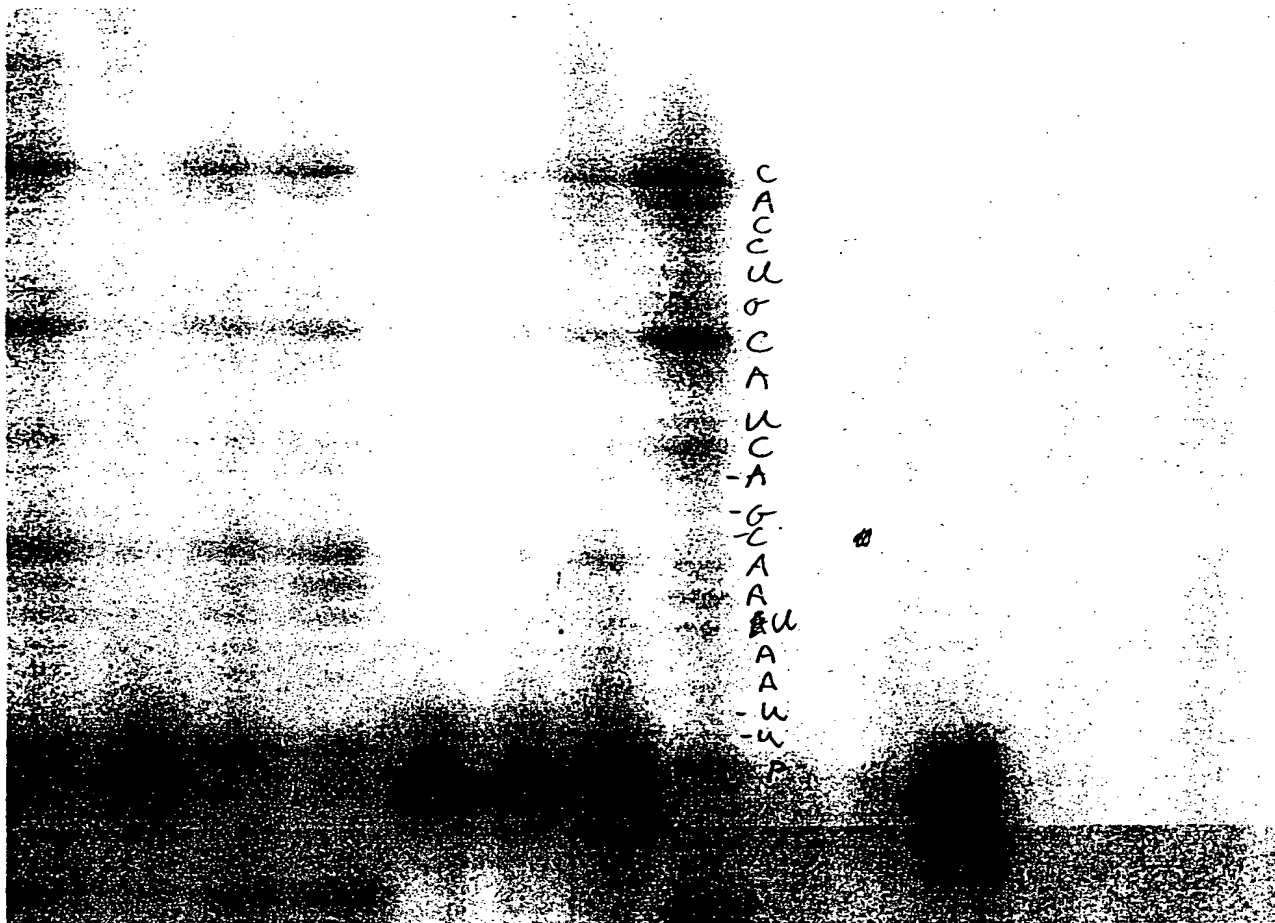
Project N \_\_\_\_\_

Book No. \_\_\_\_\_

103

ge N \_\_\_\_\_ conducting some colonies

min  
the colonies  
Tag



C  
A  
C  
C  
U  
O  
C  
A  
U  
C  
A  
-G  
-C  
A  
A  
A  
A  
U  
-U  
P

2.00 699.88

2.00x Counts

- 12/17/94 - 10:49 pm

To Page No. \_\_\_\_\_

d & Understood by m ,

Date

2/16/95

Invented by

Recorded by

Date

1-10-95

saal a Polap

ag No. \_\_\_\_\_

purpose: To minimize the overnight cultures to which  
all solutions from above

1.5 ml  $\text{D}$ 

pellet resuspended in 0.1 ml of Resuspension buffer

25 mM Tris, 8.0

50 " EDTA

1% glucose

autoclaved + stored at  $4^{\circ}$ 

added 0.2 ml of  $\text{NaOH}$  / 500 = 0.2 M  $\text{NaOH}$

on in 15

1% SDS

prepared fresh

added 0.15 ml of 7.5 M Ammonio acetate Mix + incubate 15 min

15' RT filtered + not filtered

sup + 0.9 ml of 95% ethanol Mixed well 15'

white washed with 70% ethanol 1 ml. w/o mixing 15'

again Vac dried 5'

resuspended in 25  $\mu\text{l}$ . ( $\approx 100 \text{ ng}/\lambda$ ) of TE

used 8  $\mu\text{l}$  from each prep for digestion

Reaction buffer 10x NEB 4, Afl III, Aat II & EcoRI

90  $\mu\text{l}$ 

5

2

3  $\mu\text{l}$ 

rest H<sub>2</sub>O, Volume to 192  $\mu\text{l}$

added 12  $\mu\text{l}$  / Rx. digested at  $37^{\circ}$ , 3 hrs, stored at  $4^{\circ}$

To Page No. \_\_\_\_\_

Issued &amp; Understood by me,

Date

12/16/94

Invented by

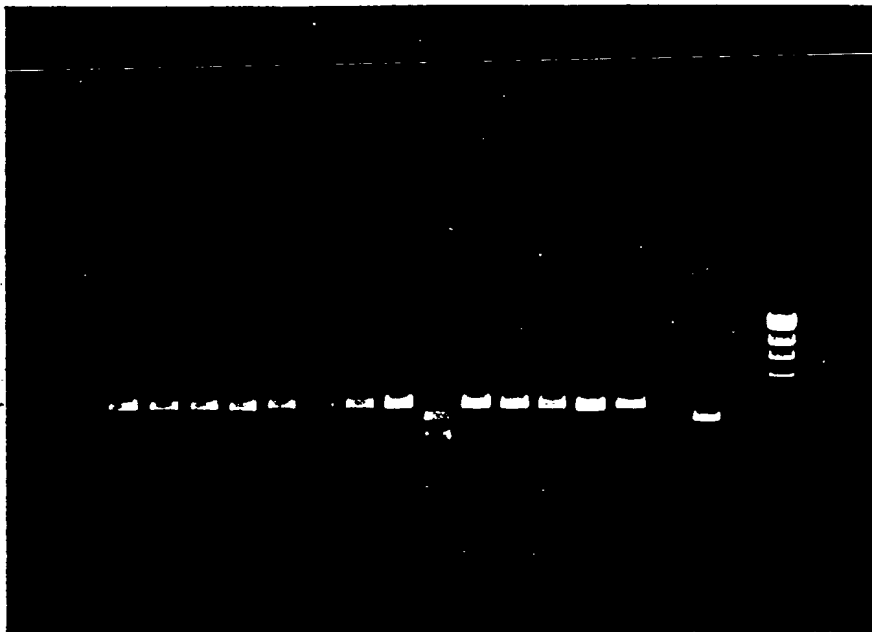
Recorded by

J. S. S. S. S.

Date

12/16/94

From Page No. \_\_\_\_\_



B W. from Tag W from T+DV

Even Palmer didn't get restricted why?

T Pag N.

Witnessed &amp; Understood by me,

Date

Invent d by

Dat

Record d by

12/22/94

Dr. Sitarman

PMCA Revisited:

Tag N .

Purpose: To repeat the exercise again with pmca for fidelity

Just :: to titrate the amount of template I used 100-200 pg / 35 cycles / 3 step - 3' extension - but of sequencing was obvious. Today has used earlier has template / 30 cycles / 2 step has of other products 5' extension

so tried 0, 10, 25, 50, 100 & 200 pg of starting template / 5' extension / 2 step

both 10 & 20 of enzyme product yield is negligible - but with 20 of Tag quite good product yield was obtained

so tried with both 10 & 20 of Tag alone

To start with, in this expt Tag, D1 wasn't included

Conditions:

10x buffer K-T.

94°, 30"

200 µM dNTP

.4 µM primers (new-bw)

30 (94°, 30", 60", 5')

2 mM Mg

Template 0-200pg

enzyme 1 & 20.

- prepared a premix 12x 45 µl

7 tubes: 1-12 10

13-24 20

- added 5 µl of different amount of template

To Page No. \_\_\_\_\_

Read & Understood by me,

Date

1/9/95

Invented by

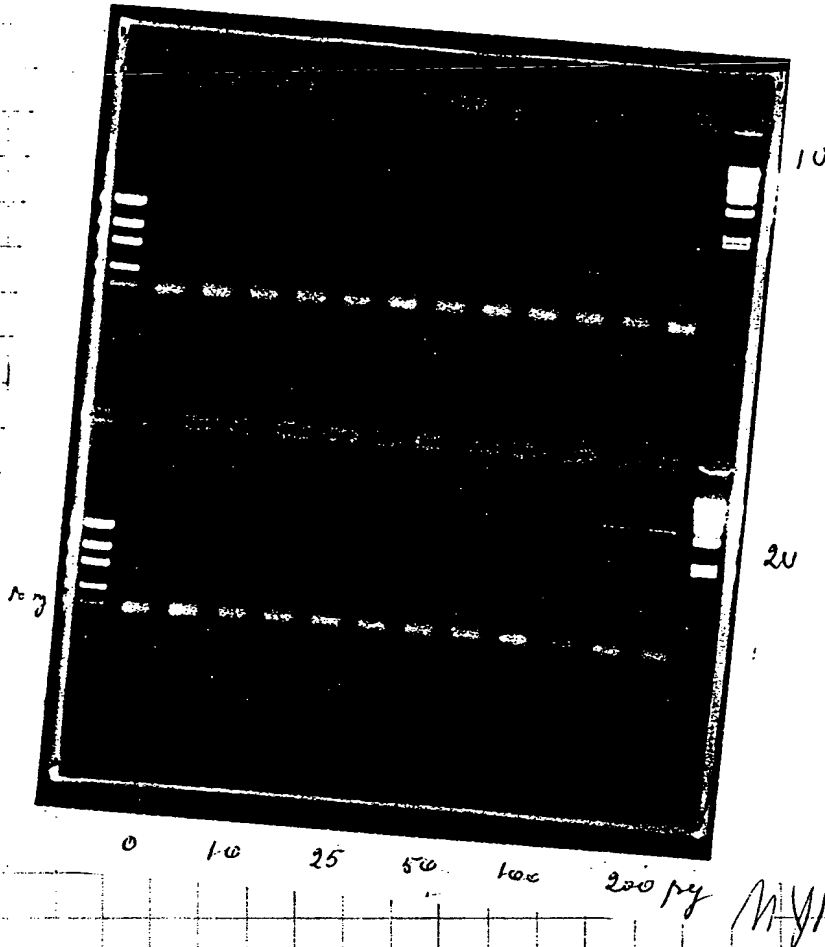
Date

Record d by

K. O'Brien

12/19/94

From Page No. \_\_\_\_\_

TagResult :

200 pg / 10 enzyme ✓

25 pg up / 20 product  
obtained but  
rather very  
yield even w. the  
200 pg of starting  
template.

~ 5 - 10 ng / spl

= 31.25 - 62.5 ng /  $\frac{1}{2}$   
from 200 pg temp

~ 300 fold ampl

~ 8 doublings !

\* Try 50 pg } 20 200 pg - 10

- cycles increased to 35 atleast

- go up to 50, 60 -

- cycling regime looks ok - gives cleaner product.

no mispriming seen.

To Page N

Witnessed &amp; Understood by me,

Date

1/9/95

Invented by

Recorded by

Dr. Sitarman

Date

12/20/94

104

Project No. \_\_\_\_\_  
Book No. \_\_\_\_\_

TITLE SDS of rTag & Native Tag prep

From Page No. repeat of 10-3-94 with high amounts of Native Tag

TCAppt. of Native Tag (see P 96, 7)

Native Tag 5  $\mu$ /pl  
lot # EPD 404  
total units  
H<sub>2</sub>O  
15% TCA (ice cold)

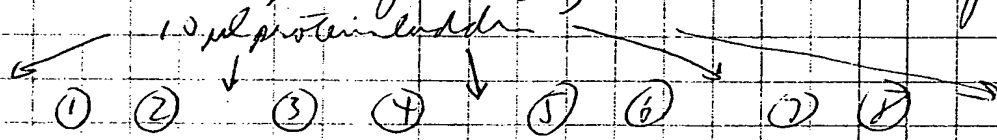
| (1) | (2)        | (3) | (4)   | (5) | (6)         | (7) | (8)         |
|-----|------------|-----|-------|-----|-------------|-----|-------------|
|     | 16         |     | 32    |     | 64          |     | 96          |
|     | (80)       |     | (160) |     | (320)       |     | (600)       |
|     | 184        |     | 168   |     | 136 $\mu$ l |     | 104 $\mu$ l |
|     | 200        |     |       |     |             |     |             |
|     | <u>400</u> |     |       |     |             |     |             |

ice 30'  
microfuge 10'  
resuspended in acetone (-20°C) stored  
microfuge 15', discard supernatant  
dry at 7°C  
resuspended in 50  $\mu$ l 1X sample buffer

rTag EKRT1 lot #  
dilute 1/4 in 1X crash  
(now its 80%  $\mu$ l)

|                  |    |    |    |    |
|------------------|----|----|----|----|
| 1X sample buffer | 50 | 48 | 46 | 44 |
|------------------|----|----|----|----|

Load all 50  $\mu$ l after 5', 90°C as follows



gel same as P 140, 6

1.5 min spacer  
1X wells

start 10:10 AM at 28 mA (= 72 V)  
(maintain ~ 30 mA constant)

room temp same as P 148, 6

stopped gel at 3:20 5 hr 10 min total time To Page N

Witnessed & Understood by me,

Deena Pokorski

Date

2/16/95

Invented by

Recorded by

Date

1-11-95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

miniups for Aeyou's plates  
of 1-10.95 0, .05, 0.1 mM Mn

106

From Page No. \_\_\_\_\_

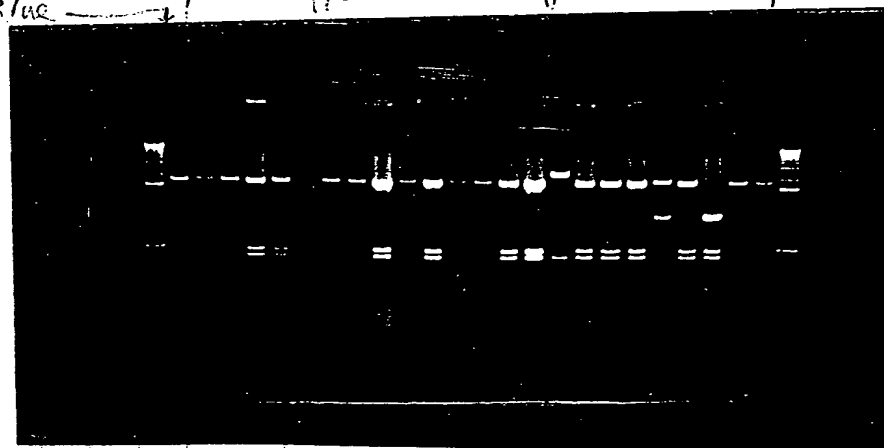
for plate PCR

grow O/N, 2 ml card grow, 10<sup>6</sup> pg/ml

|                         |      |       |        |     |
|-------------------------|------|-------|--------|-----|
| mM Mn Cl <sub>2</sub> = | 0    | 1     | Blue   | (1) |
|                         | 0    | 2-6   | whites | (5) |
|                         | 0.05 | 7-15  | whites | (9) |
|                         | 0.1  | 16-24 | whites | 9   |

best same as P.93. 2 hr 37°C Aat II, Afl III, Ew R, NEB by

Mn++ 0 0.05 mM 0.1 mM  
Blue →



Aat/R1  
465bp →  
410bp →  
Afl III/R1

Aat II 46

Afl III  
183  
puc19  
2676

↑  
- Aat II  
only  
410bp  
Afl III/R1  
bands  
present

↑  
- R1 in #25  
R1 is missing  
875  
Aat ↑ Afl  
R1

Results:

for 0.1 mM Mn Cl<sub>2</sub> 3 of 9 are rearrangements;  
2 are in loc region, one is in vector

0 and 0.05 mM Mn are 9/9 full length

To Page N

Witnessed &amp; Understood by me,

Deerana Polans

Date

2/16/95

Invented by

Recorded by

Date

1-12-94

Page No. \_\_\_\_\_

Purpose: Amplification of pMC9 with 20  $\mu$ g Tag and different amount of Deep Vent.

prepared mixture with 20  $\mu$ g Tag w/o any Deep Vent.

added dif. amount of Deep Vent, done in duplicate.

| Tag | D.V  | $\mu$ l |
|-----|------|---------|
| 20  | 5    | 2.5     |
|     | 2    | 1       |
|     | 1    | 0.5     |
|     | .5   | 0.25    |
|     | .2   | 2       |
|     | .1   | 1       |
|     | .05  | .5      |
|     | .02  | 2       |
|     | .01  | 1       |
|     | .005 | .5      |
|     | .001 | 1       |
|     | 0    | 0       |

$$1 \mu\text{l} \rightarrow 20 \mu\text{l} = 0.1 \text{ U} / \mu\text{l}$$

$$> 10.01 \text{ U} \leftarrow 1/10$$

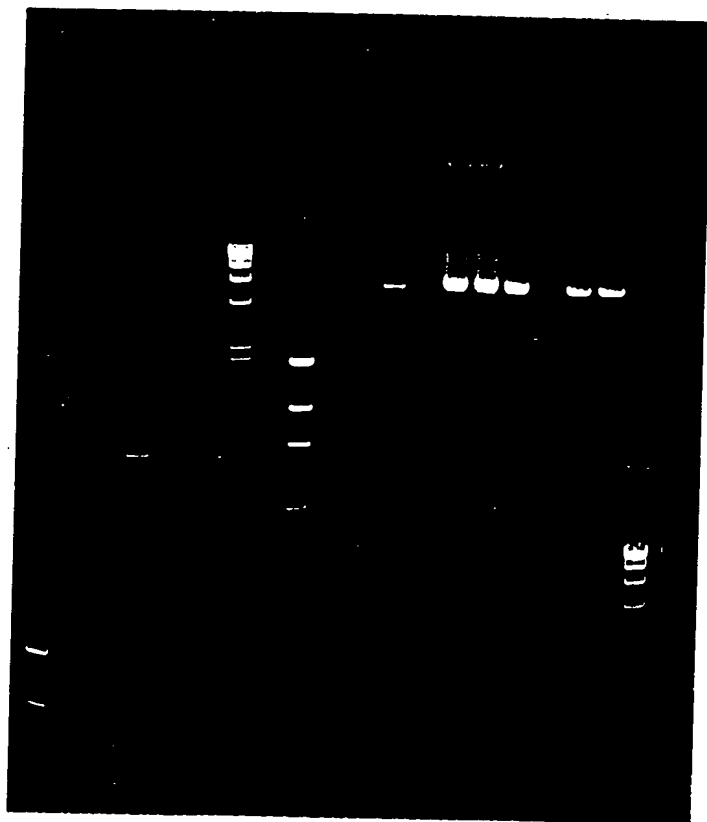
$$1/10$$

200  $\mu$ l dNTP  
4  $\mu$ l primer  
50  $\mu$ g Template  
2 mM Mg

94° 30"  
(94° 30")  
68° 5' 85

|                  |                        |
|------------------|------------------------|
| H <sub>2</sub> O | 1120                   |
| x buffer         | 140                    |
| Tag              | 28                     |
| dNTP             | 28                     |
| mixture 1        | 10.5                   |
| 2                | 11.8                   |
| Template         | 56.0 (50 $\mu$ g / rx) |
| Tag              | 5.6                    |
|                  | 1400                   |

has to be repeated again  
low into bottom.  
pink Rxs too.



Prepared &amp; Understood by m ,

Date

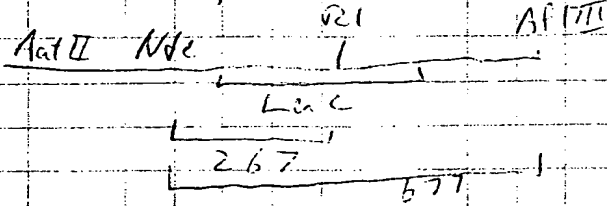
Inventor

R cord

Dr. Subraman

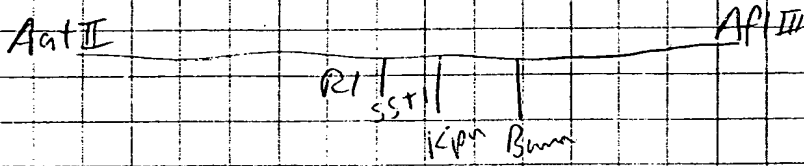
12/22/94

age N — cut on at Nde I to see if all of loc is present even though Aat II is missing



Nde I is OK in buffer 4 (NEB) (P169)  
 expect 677 bp Nde I / Afl III band

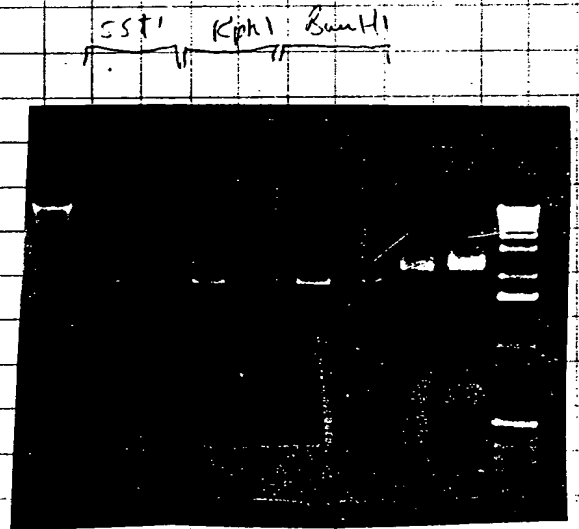
cut with Sst I, Kpn I or Bam HI to see if missing, R1 site is only a point mutation, (all OK in NEB buffer 4)



(for cloning #20  
 (tubes 2, 4, 6 below)  
 see if Sst I, Kpn I or Bam  
 can cut in MCS

tube # 1 2 3 4 5 6 7 8

|                |       |   |   |   |   |
|----------------|-------|---|---|---|---|
| A #1           | 5     | 5 | 5 | 5 | ✓ |
| (no cloning)   |       |   |   |   |   |
| missing R1     | 5     | 5 | 5 |   | ✓ |
| missing Aat II |       |   |   | 5 | ✓ |
| + II           | 0.1   | → | → | → | ✓ |
| I III          | 0.3   | → | → | → | ✓ |
| I              | 1     | 1 |   |   |   |
| I              |       | 1 | 1 |   |   |
| HI             |       |   | 1 | 1 |   |
| 21             |       |   |   | 1 | 1 |
| 20             | 11.6  | → | → | → | ✓ |
| buffer 4       | 2     | → | → | → | ✓ |
| mult.          | 20 µl |   |   |   |   |

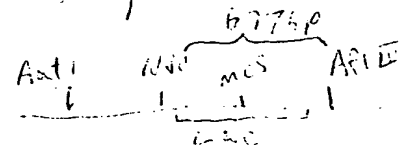


37°C 2 hr

G A A T T C G A G C T C  
 R1 Sst I

point mutation

Sst I cuts but R1 did not (P106)  
 is mutation is no more than 1 bp downstream  
 from the R1 site and may even be a



To Page No. \_\_\_\_\_

We may give the 677 bp fragment expected  
 if multiple bands are seen

sed & Understood by me,

Ernesta Bokor

Date

2/16/95

Invented by

Recorded by

Date

1/13/95

Project No. \_\_\_\_\_

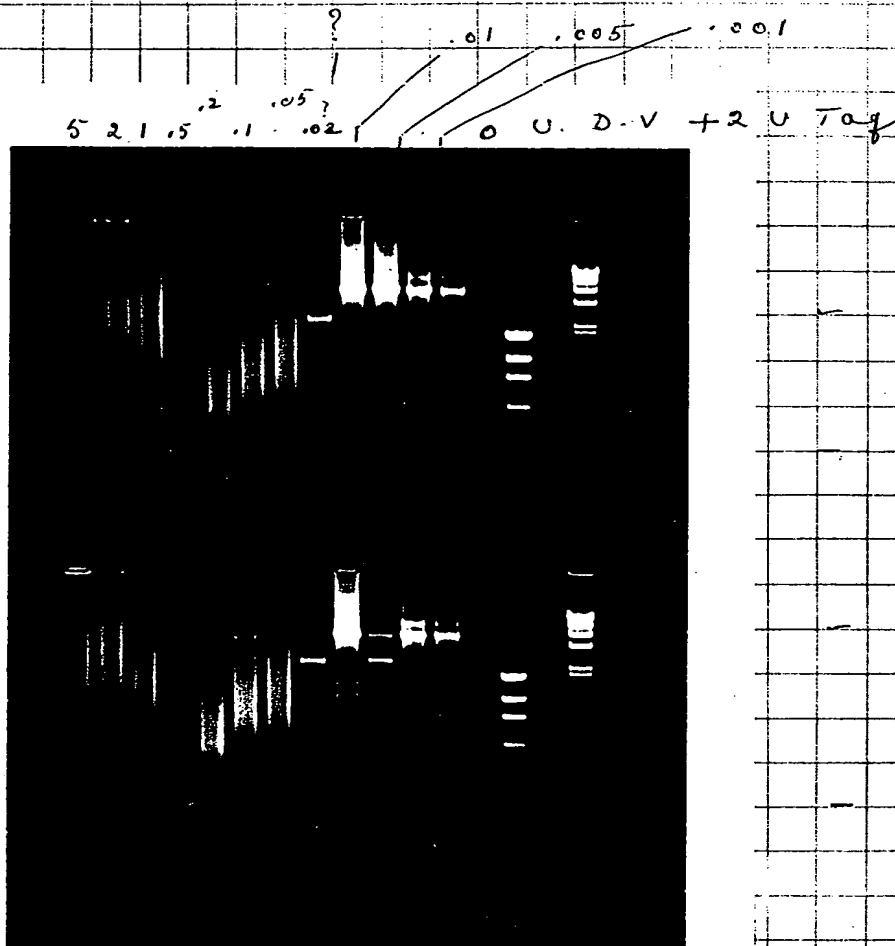
144 12/27/94

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

From Page No. \_\_\_\_\_

The gal. was run.



Result:

Tag 20 / 50 pg & yield

mispriming still under these conditions

Higher conc of Dap in the presence of Tag no product

0.02 U + 2 U Tag no product either

but with 0.01 U + 20g Tag plenty of product

Is this Dap vent too, or mistake in diluting??

check again w. new Rx.

To Page

Witnessed & Understood by me,

Date

Invented by

Date

Record d by

K. Blahman

12/28/94



34P 733

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

109

Page No. — Same as P. 99

make 40  $\mu$ l of 32P 733

① (JT)

20  $\mu$ l

② (JA)

20  $\mu$ l2863  
or #678 (JT)  
10 pmol/ $\mu$ l5.05  $\mu$ l

✓

#678 (JA)

7.5  $\mu$ l

✓

6.76 pmol/ $\mu$ l

NTsio pH 7.5

2

2

✓

H<sub>2</sub>O

40

56.5

✓

V<sub>A</sub> = 66

5'; 70°C → cool slowly

To Page No. \_\_\_\_\_

Designed &amp; Understood by me,

Date

Invented by

Date

Sandra Polansky

2/16/95

Recorded by

176-95

Page No. \_\_\_\_\_

upstream: PCR amplification with 20g enzyme +  
different amount of Deep Vent.

Repeat of previous expt, 4 of points below.

200  $\mu$ M dNTP

D.V.:

0.4  $\mu$ M primers

50 pg Template

2 mM Mg

2 U Tag

1, 0.5, 0.2, 0.1, 0.05, 0.02, 0.01,  
0.005, 0.002, 0.001, 0

0.1% diluted to 0.101%  $\rightarrow$  1/10 = 0.0101%  $\rightarrow$  1/10 = 0.00101%  
in 1x buffer w/o Mg.

prepared premix 25x, done in duplicate.

45  $\mu$ l of " + 5  $\mu$ l of different amount of enzyme.

H<sub>2</sub>O

10x buffer

125  $\mu$ l

dNTP 10mM

25

Mg 100mM

25

primer 1

10.6

2

9.5

Template

25.0

1125  $\leftarrow$  added 2.5  $\mu$ l Tag = 250

removed 40  $\mu$ l = w/o any enzyme

After adding Tag, mixed & aliquoted 45  $\mu$ l / rx to diff. tubes

added Deep Vent diluted different conc.

To Page N \_\_\_\_\_

Seen &amp; Understood by m ,

Date

1/9/95

Invented by

Date

Recorded by

K. S. S. S. S.

12/27/94

1122

|           |    |    |    |   |   |    |    |    |
|-----------|----|----|----|---|---|----|----|----|
| 207 Toy + | 0  | 1  | 5  | 2 | 1 | 05 | 02 | 08 |
| 0         | 15 | 13 | 11 | 9 | 7 | 5  | ✓  |    |

20 Tagt 3 17 19 21  
101 105 102 100108 DV

Recorded by \_\_\_\_\_

12/28/94

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

110

From Page No. \_\_\_\_\_

① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩ ⑪ ⑫

32P 733 .2863  
(dT)

5 —————→

32P 733 .678  
(dT)

5 —————→

10 x Vent buffer  
4 JNTPA 10 mM each  
dCT, G-TP 2.5 mM each5  $\mu$ l1  $\mu$ l

44

rTag 3  $\mu$ l EKBTI  
dilute to 0.25 units/ $\mu$ l

2 2 2 2 2 - 2 2 2 2 -

\* Vent DNA polymerase

0.125  $\mu$ l

1

0.5  $\mu$ l

1

2  $\mu$ l

1

1

1

1

1

1

H<sub>2</sub>O

34 35 37

50  $\mu$ l

39

37

35

70°C, remove 10  $\mu$ l to 5  $\mu$ l stop at 2, 5, 10 min

pol mix

rTag 3  $\mu$ l

2

20.67 1.85 0.5

Vent 2  $\mu$ l

\*

1

2

3

\*

Tag storage buffer

22

44.3 20.67 5.1

1/2 27

48 24 9

✓

Add

2 1

3

3

3

1

note ix vent buffer is 2 mM MgSO<sub>4</sub>

\* dilute with Vent dil storage buffer

To Page N

Witness d &amp; Understood by me,

Date

Invented by

Date

Deanne Bolamp

2/16/95

Record d by

1-17-95

Tag No. \_\_\_\_\_

purpose: To check again pMC9 w/ Deep vent } alone  
Tag

50  $\mu$ g Template  
200  $\mu$ M dNTP  
1.4  $\mu$ M primer  
2 mM Mg

prepared premix 45  $\mu$ l / rx  
added diluted enzyme in 5  $\mu$ l

for DV tried: .5 u.

.2

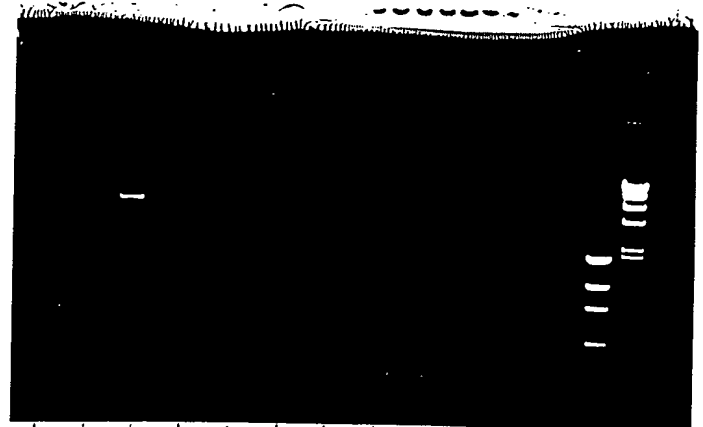
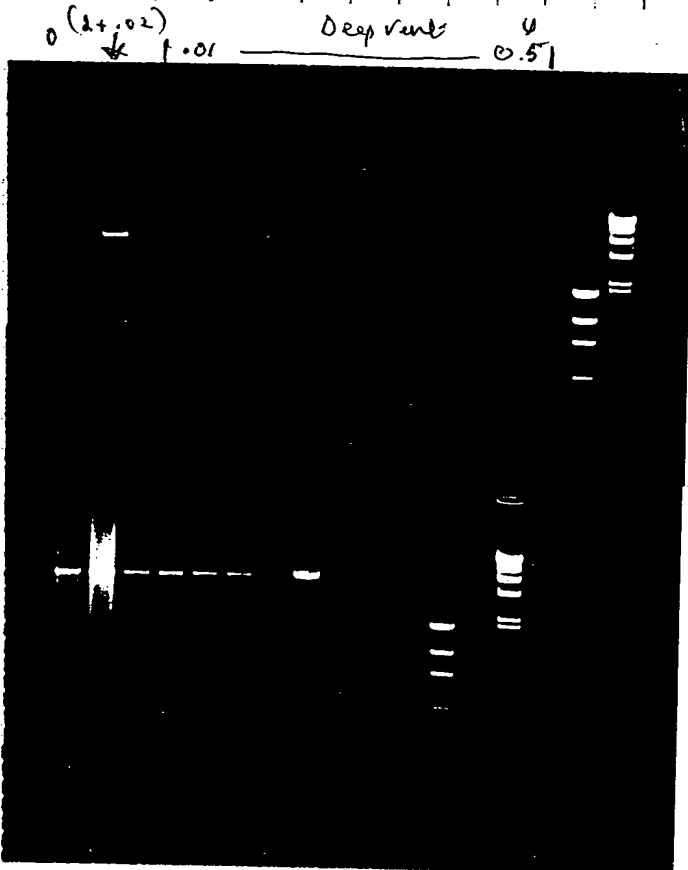
.1

.05

.01

for Tag: 5, 2, 1.5, 0

Mix: (2+.02) and (1+.01)



4 5 2 1.5 0 (1+.01)  
(2+.02) mix

Increasing less than what  
con of Tag one would expect  
increased product  
yield.

↑ order?

To Page No. \_\_\_\_\_

Used &amp; Understood by me,

Date

Invented by

Date

Recorded by

12/28/94

K. Starnan

# SDMTP sequencing reactions

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

Exhibit 30  
Appl. No. 09/558,421

111

| Tube N   | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 55       |
|----------|----|----|----|----|----|----|----|----|----------|
|          | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |          |
|          | 1  |    |    |    |    |    |    |    | ✓        |
| buffer 1 |    |    |    |    | 1  |    |    |    | ✓ primer |
| mix      | 2  |    |    |    | 2  |    |    |    | ✓        |
|          |    | 2  |    |    |    | 2  |    |    | ✓        |
|          |    |    | 2  |    |    |    | 2  |    | ✓        |
|          |    |    |    | 2  |    |    |    | 2  | ✓        |
| 5.5      |    |    |    |    |    |    |    |    | ✓        |
| 4.5      |    |    |    |    |    |    |    |    | ✓        |
| 0.5      |    |    |    |    |    |    |    |    | ✓        |
| 10 µl    |    |    |    |    |    |    |    |    | ✓        |

5 min, 70°C, → add 5 µl stop

Sequencing see P 27, 4

|                               |         |         |   |
|-------------------------------|---------|---------|---|
| <sup>32</sup> P 733-2863 (dT) | 12      |         | ✓ |
| P 733-677 (dA)                |         | 12      | ✓ |
| DTT 0.1M                      | 1       | 1       |   |
| sequencing kit buffer         | 2.27    | 2.27    | ✓ |
|                               | 2.23    | 2.27    | ✓ |
|                               | 17.5    | 17.5    |   |
| 3.5                           | ✓       | ✓       | ✓ |
| AT C G T                      | A C G T | A C G T |   |

Sequencing 2.5 µl  
0.2

To Page No. \_\_\_\_\_

Read & Understood by me,

*Erin Polansky*

Date

2/16/95

Invented by

Recorded by

Date- 17.55

1-18-95

**TITLE**

Purpose:

Hg titration in pcring pMCP with Tag  
and Tag + D.V.

did at 200 mg d nTP.

14 May summer  
30 May timetable

Mg : 1, 1.5, 2, 2.5, 3 mM

$$(T_1 - T_3) \quad 2N$$

$(2 \pm 0.02)$  numbered

prepared premix with  $\text{Ta}_2\text{O}_5$  or  $\text{Ta}_2\text{O}_5 + \text{O}_2$  separately 4.57

added diff amount of Mg in spl

same cycling conditions

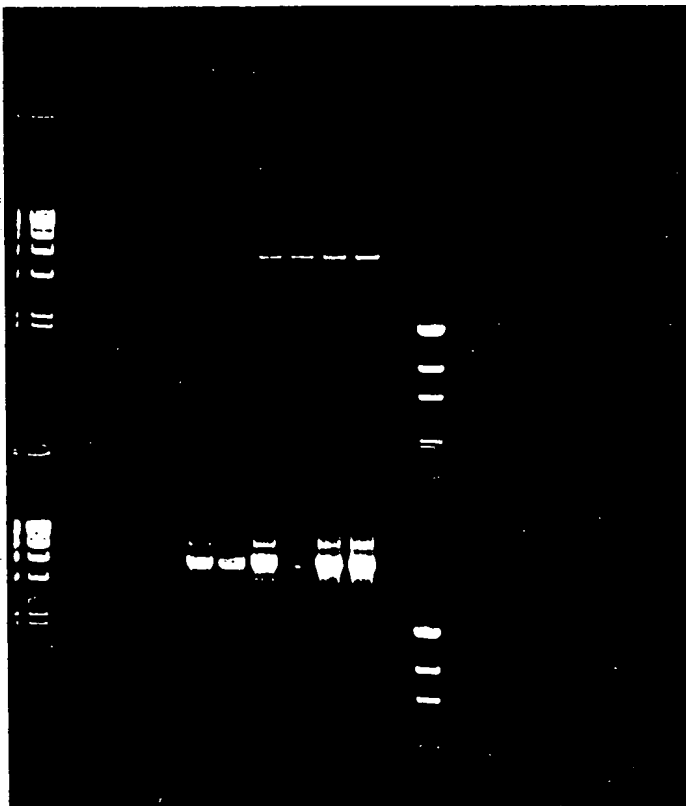
Result :

20 g Tag : increase  
the Chem of Mg for 2.5  
3

2 V considerably since  
the products yield.

2V. Tcy = 0.02 V DV  
 even at 1.5 mV Mg  
 product seen.

more produced as  
as more misprints  
with increasing con  
ng.



1 1.5 2 2.5 3 mV Tag.  $\pm 0.02$   
2  $\pm 0.02$

12/28/14

rk. Abraham

**T Page**

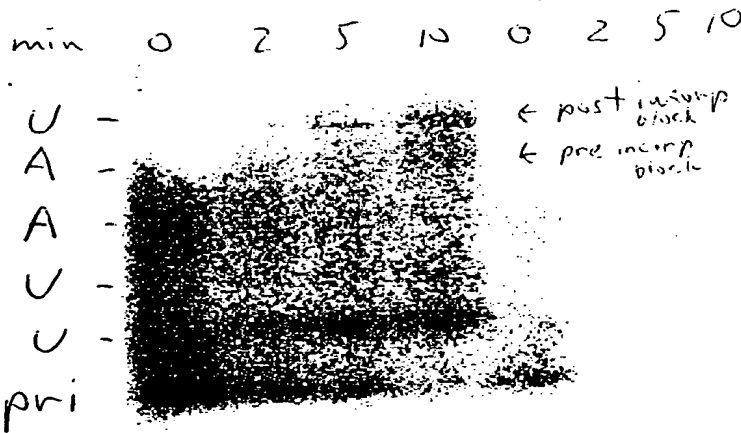
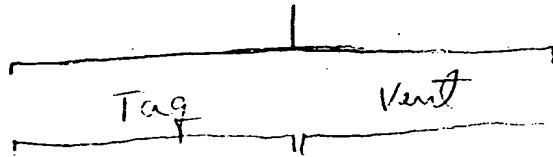
DU.GEL

- 01/19/95 - 09:20 pm

2.00x Counts

29.99  200.

- dATP and (T) primer



← primer degraded

A gel electrophoresis image showing a single band labeled 'primer degraded'.

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T P

Page No. \_\_\_\_\_

upset: To check 1. Tag dil amount (since 1.50 works in one case)  
 2. mix of (Tag + DV)  
 against

3. freshly made (added separately)

| Tube | U of Tag    | # Tube | (T + DV)          |
|------|-------------|--------|-------------------|
| 2    | 5) left out | 13 14  | .5 ml .50 + .0050 |
| 4    | 2.5         | 15 16  | 1 1 + .01         |
| 6    | 2           | 17 18  | 1.5 1.5 + .015    |
| 8    | 1.5         | 19 20  | 2 2 + .02         |
| 10   | 1           | 21 22  | 5 5 + .05         |
| 11   | 0           |        |                   |
|      | ↑           |        |                   |

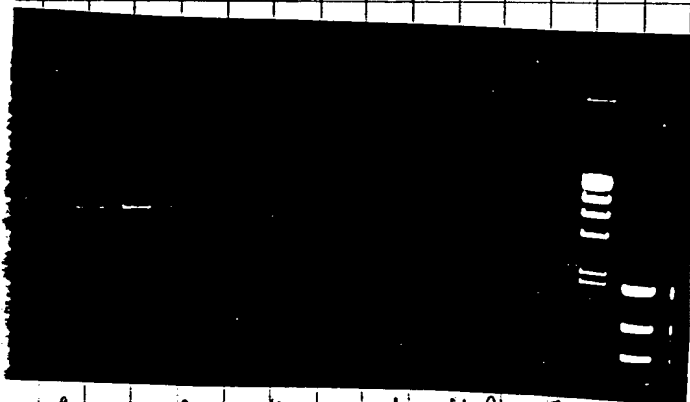
(added in 5ml +) 45ml rx (+ added in 5ml)

under cycling conditions.

10 Tag + D.V. .005, .01, .020 - diluted in 5ml.  
 20 Tag + D.V. " " "

Result: w. Tag above  
 10 barely results in  
 product.

- frequent freeze-thawing?  
 (



To Page No. \_\_\_\_\_

ed &amp; Und rst od by m ,

Date

Inv nt d by

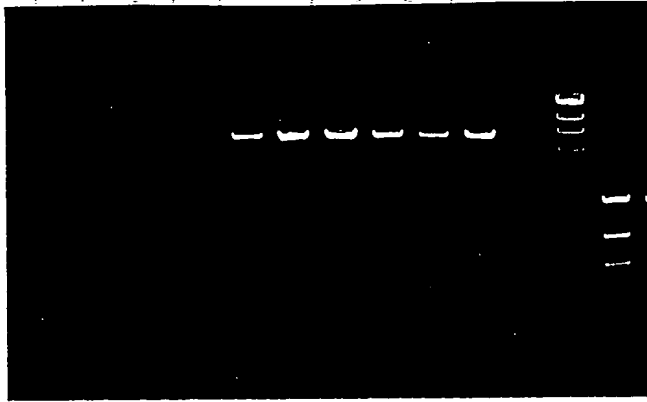
Date

Recorded by

J. Silvarman

1/3/95

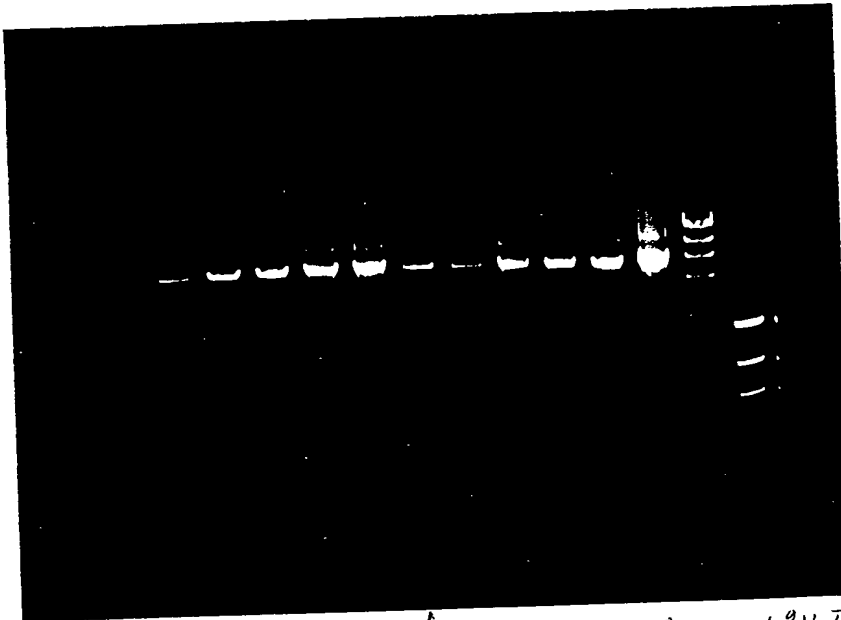
premix:



0.5 1 1.5 2 5 11.91

↓ repeated <sup>to</sup> again - looks ok

fresh mix:



10 Tag + 0.005 1.01 0.020 1.005 1.01 1.02 + 20 Tag

2 + .02 - 1 + .01

*per*

*CU*

- Ruth with 10  
 level of pay  
 increasing the  
 amounts of D  
 from 0.005  
 to 0.2  
 the feedback  
 gets up!

manix seen  
he holding

**To Page 1**

*K. Skrammen*

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_ TITLE \_\_\_\_\_

Miniprep for Ayoabs  
PCR

114

From Page No. \_\_\_\_\_

miniprep #

Ayoabs PCR conditions

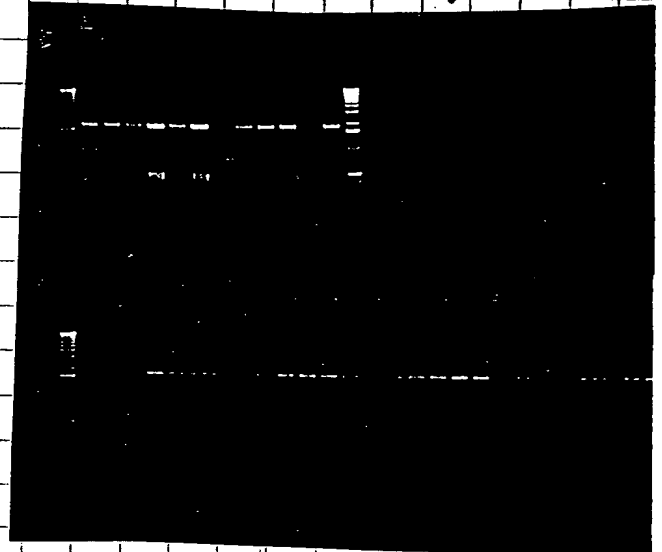
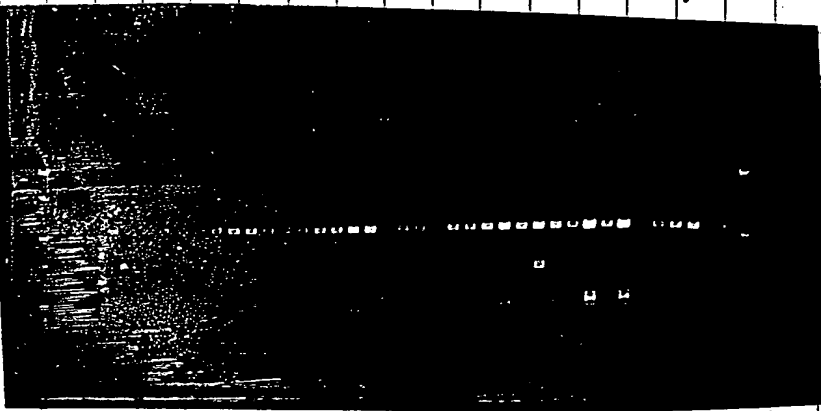
| miniprep # | Tag | Tag + Deep Vial | W/N |
|------------|-----|-----------------|-----|
| # 1-20     | +   |                 | 0   |
| 21-40      | +   |                 | .05 |
| 41-60      | +   |                 | 0.1 |
| 61-80      |     | +               | 0   |
| 81-100     |     | +               | .05 |
| 101-120    |     | +               | 0.1 |
| 121        |     | Blue colony     |     |

grow O/N 30°C, 2 ml circle grow + 100 µg/ml Amp

miniprep same as p41, 4 using 1 ml cells

digest as per P 93 A1FII, Aat II, Eco RI 5 µl miniprep  
1-40 on 42 well comb, load 10 µl  
conclude resolution not good  
enough for ~500 bp range

1-40 on 30 tooth comb, load  
need more DNA in digest and load 2



To Page N

Witnessed & Understood by me,

Deanna Polanco

Date

2/16/95

Invented by

Recorded by

Date

127-15  
30-15-12A

**Book No.**\_\_\_\_\_

1/3 - 4/95 151

sealed (1) 1 + .01 (duplicates) } 12/25/94  
 (1) 2 + .02 " }  
 (4) 2 + .1 duplicates } 12/27/94  
 (3) 2 + .01 " }  
 (2) 2 + .001 " }  
 Tag alone 2 v } duplicate 12/28/94  
 1 v } duplicate 12/28/94 } 200 pg ~~trans~~ photo  
 phenol / chloroform extracted 1x  
 ethanol pptd under night  
 resuspended in 25  $\mu$ l of 1x TG - all different amount - can  
 not check con  
 all 7 samples given to Judy Owen 1/4/95

~~sed & Understood~~ by me,

**Date**

**Invented by**

**Date**

**To Page No.\_\_\_\_\_**

**Recorded by**

175795

115

digest 2hr 37°C load 20  $\mu$ l

$$V_p = 10 \mu\text{V}$$

Agist 10 ml in 20 ml  
 $V_f = 20 \text{ ml}$

*[Handwritten signature]*

57-81

4-29-56

← 1-2A

05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100

$$= 44$$

↑ ↑ ?  
O2L ? ↑ ?

see full length  
for #13  
see P123

**To Page No.\_\_\_\_\_**

**Date**

1-30-95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

152

1/3/95

From Pag No. \_\_\_\_\_

## Applications:

### Amplification from plasmids:

Purpose: - To start the cultures of different size fragments in pDELTA 1

- all glycerol stocks obtained from this young

|        |     |    |                           |
|--------|-----|----|---------------------------|
| 6.4 Kb | PYA | 21 | 3 ml of LB + 100 µg/ml    |
| 8.0    |     | 57 | freshly made Amp          |
| 10.5   |     | 20 | a stock of glycerol stock |
| 20.0   |     | 47 | overnight at 37°          |
| 29.0   |     | 17 |                           |

1/4/95

- except for # PYA 20 - 10.5 Kb rest of them grew quite well

- It was regrown again overnight with fresh stock.

- Rest of them 1.5 ml of each was miniprep. using alkaline lysis method. see page 139. stored at 4° suspended in 25 µl of 1X TE.

- Each culture was diluted 10 + 990 (100) - 1/100 dilution plated 25 µl of diluted culture onto per made Amp plates, incubated at 37°, overnight

6.4 + # 57 and # 21 were overgrown but good isolated colonies in the periphery of the plate.

21 # 17 gave just 4 colonies in 2 plates!

20 # 47 quite a few with spread, full blown large colonies

- Replated further diluted # 57 + 21 for further use.

To Pag

Witness & Understood by m ,

Dat

Inv nt d by

Dat

Recorded by

R. Satharaman

1/5/95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

Results P 115

116

From Page No. \_\_\_\_\_

|                                   |       |                  |   |   |   |
|-----------------------------------|-------|------------------|---|---|---|
| #1-20, Tag 0 Mn                   | 17 19 | (see P 123 SSTI) | 1 | X | - |
| 21-40, Tag 0.5 mM Mn              | 18 20 |                  |   | X | - |
| 41-60, Tag 0.1 mM Mn              | 18 2  |                  |   |   |   |
| 61-80, Tag 0 Mn<br>+D Vent        | 12 17 | X                | 1 | 2 | X |
| 81-100, Tag 0.5 mM Mn<br>+D Vent  | 18 2  |                  |   |   |   |
| 101-120, Tag 0.1 mM Mn<br>+D Vent | 16 4  |                  |   |   |   |

see new  
Table on P 124  
after Ord I and SST  
cuts

SEE NEW  
Table on P 124  
after DrdII and SST  
cuts

- (\*) gDomes lacks RI site in mcs  
(F) no result, i.e. not enough DNA  
to be sure about cut.

confirmed deletions  
miniprep #19, 61, 65

0.1 mM Mn, Tag + Deep Vent



only 410 or 465 removed  
so it's 2.2 bp

1.8 bp  
has 410 and 465 removed

miniprep #

Witnessed &amp; Understood by me,

Deena Polansky

Date

2/16/95

Invented by

Recorded by

Dat

1-31-95

P116 continued  
Experiment done on P. 123

Project N. \_\_\_\_\_  
Book N. \_\_\_\_\_

117

ag No. \_\_\_\_\_

Still Needed 8

cut with Orf I to see if full length lac Z is present  
(assuming either Afl III or Aat II recognition region  
had a point mutation/generation). Therefore the "410" and "465" bp

miniprep # 54, 58, 64, 73, 87, 98, 103, 108, 113, 120

plus Aat II, Afl III

cut with Sst I to see if R1 site in MCS was  
a point mutation (or very small deletion  
(all on P107 at bottom) resulting in the "90mers"

miniprep # 3, 29

Recut with 17  $\mu$ l miniprep and load 30  $\mu$ l?  
2.5  $\mu$ l reaction

to try to resolve the "No results"

miniprep # 20, 39, 71, 74, 75, 76

To Page No. \_\_\_\_\_

Read & Understood by me,

\_\_\_\_\_

Date

2/16/95

Invented by

Recorded by

Date

1-31-95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE *Work at Frederick*

From Page No. \_\_\_\_\_

|         | SN | CPM      | TIME |
|---------|----|----------|------|
| A1      | 1  | 4976.00  | 0.50 |
|         | 2  | 3216.00  | 0.50 |
|         | 3  | 4500.00  | 0.50 |
| A0      | 4  | 16920.00 | 0.50 |
|         | 5  | 17020.00 | 0.50 |
|         | 6  | 16156.00 | 0.50 |
| A1      | 7  | 3926.00  | 0.50 |
|         | 8  | 3822.00  | 0.50 |
|         | 9  | 4054.00  | 0.50 |
| A2      | 10 | 15974.00 | 0.50 |
|         | 11 | 16520.00 | 0.50 |
|         | 12 | 15478.00 | 0.50 |
| A3      | 13 | 4684.00  | 0.50 |
|         | 14 | 4752.00  | 0.50 |
|         | 15 | 4606.00  | 0.50 |
| A0      | 16 | 17622.00 | 0.50 |
|         | 17 | 16806.00 | 0.50 |
|         | 18 | 17742.00 | 0.50 |
| LTI     | 19 | 4186.00  | 0.50 |
|         | 20 | 3966.00  | 0.50 |
|         | 21 | 3986.00  | 0.50 |
| LTI     | 22 | 14842.00 | 0.50 |
|         | 23 | 14704.00 | 0.50 |
|         | 24 | 15620.00 | 0.50 |
| L       | 25 | 4458.00  | 0.50 |
|         | 26 | 4644.00  | 0.50 |
|         | 27 | 3970.00  | 0.50 |
| L       | 28 | 16730.00 | 0.50 |
|         | 29 | 16914.00 | 0.50 |
|         | 30 | 15684.00 | 0.50 |
| L3      | 31 | 4864.00  | 0.50 |
|         | 32 | 5020.00  | 0.50 |
|         | 33 | 4538.00  | 0.50 |
| L3      | 34 | 15236.00 | 0.50 |
|         | 35 | 17922.00 | 0.50 |
|         | 36 | 17898.00 | 0.50 |
| Aguasol | 37 | 12.00    | 0.50 |
|         | 38 | 16.00    | 0.50 |
|         | 39 | 16.00    | 0.50 |

delivered 10µl with p10 (wiped tip)  
rinse 3x into 4 ml aguasol

each dilution had 3µl of 1µCi/ml <sup>3</sup>H TTP

To Page 1

Witnessed &amp; Understood by me,

Date

Invented by

Date

*Deanna Polansky*

2/16/95

Recorded by

1/25/95

New rTag dilutions

g N \_\_\_\_\_

#

EKBT1

77.4

18.6  $\mu$ l323 units/ $\mu$ l (P91)

Tag dilution buffer

4922.6  $\mu$ l1981.4  $\mu$ l $V_f = 5$  ml  
(5 units/ $\mu$ l) $V_f = 2$  ml  
(3 units/ $\mu$ l)

both are labelled "1-31-95 rTag"

To Page No. \_\_\_\_\_

s d &amp; Und rstood by me,

Dat

Inv nt d by

Dat

Deena Solari

2/16/95

R c rded by

1-31-95

6.4 kb

Page No. \_\_\_\_\_

purpose: To amplify 6.4 kb and 8.0 kb from plasmid

used F + R (non do) primers

50 µl rx. 200 µM dNTP each

.4 µM primers

2 mM Mg

Template ?

1 µl enzyme pre mixed

used buffer B.

Cycling: 94°; 1'

94° 30"  
60° 45"  
72° 3"

prepared enough premix for 20 rx:

6.4 kb:

all done in duplicate.

included purified prep at a known concentration

con. tried 50 pg & 100 pg  
(tag 50) just one.

mini prepped, unknown concentration (from the amount of colonies in 1/100 dilution)

con. should be quite high in the mini prep. diluted to 50 µl

used .5 µl and 1 µl

plasmid - picked a single isolated colony directly into the reaction mix containing all the rest of the stuff done in duplicate

8.0

no purified stuff available

mini prep

unknown con.

lot of colonies

from 1/100 → 25 µl dilution

.5 and 1 µl

(out of 60 µl from 1.5 ml culture)

plasmid

2

one in each

done in duplicate

T Page No. \_\_\_\_\_

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Dat

1/9/95

Inv nt d by

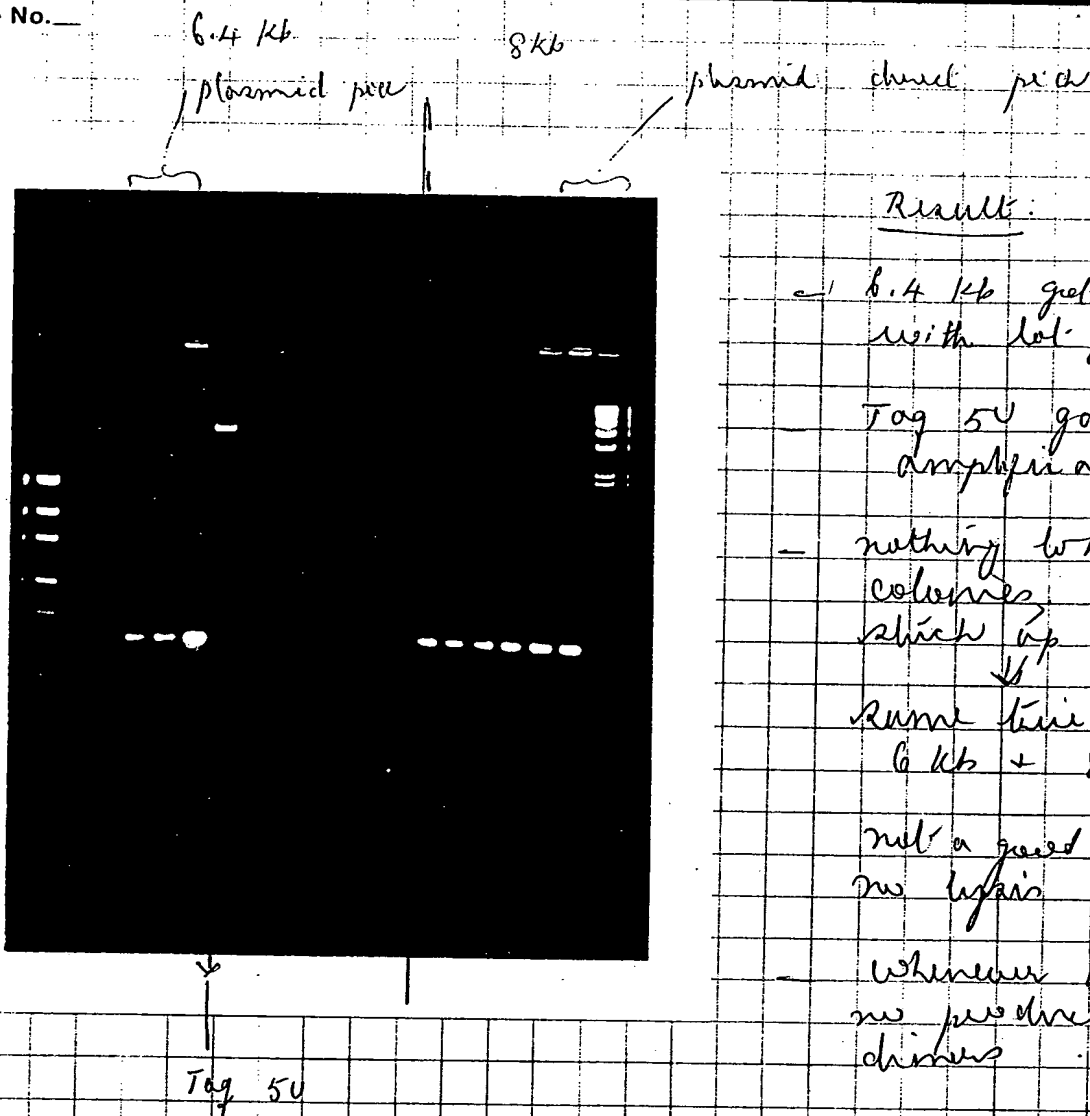
Recorded by

J. Silaraman

Dat

1/5/95

From Page No. \_\_\_\_\_

Results:

- 6.4 kb got amplified with lot of mispriming

Tag 50 gave good amplification

- nothing to be seen for colonies, lot of stuff stuck up in the well

Same time with both 6 kb + 8 kb

not a good way w/ no lysis at all,

- whenever there were no products lots of products

- amount of primers, to be found enough.

\* check alternate cycling conditions to get rid of mis priming

\* lysis in PK and just water has been checked next

\* make 6.4 kb to work first

T Page No.

Witnessed &amp; Understood by me,

Date

Invited by

Date

Recorded by

Dr. Subramaniam

1/9/95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

Accuracy of delivering 1  $\mu$ l  
with P2 pipetman for Tag Storage

From Page No. \_\_\_\_\_

add 1  $\mu$ l, 10 times to a weigh boat with  
a drop of H<sub>2</sub>O in it so tip can be rinsed  
several times. Use storage buffer at 0°C (on ice)

add H<sub>2</sub>O Tare 0.00001  $\mu$ l

2

3

4

5

6

7

8

9

10

0.0119

 $(\frac{94}{100}) =$ 

0.011

note 10  $\mu$ l SB = 0.01

.0094

3.0  $\sim$  1.1  $\mu$ l was added instead of the 1  $\mu$ l intended

conclude ~~2  $\mu$ l~~ 1  $\mu$ l is OK to add to unit assay

conclude 2  $\mu$ l is better to add for units

stock for Tag unit assay use

3 ml 0.5 M TAPS pH 7.3 150  $\mu$ l120  $\mu$ l 1 M MgCl<sub>2</sub> 6  $\mu$ l1 ml 3 M KCl 50  $\mu$ l

VP = 206

CP in unit assay

25 mM  $\rightarrow$ 2 mM  $\checkmark$ 50 mM  $\checkmark$ 

for 6607 Rxs

32P & CTP 10  $\mu$ l  $\checkmark$ 0.1 M OTT  $\checkmark$  310 mM DKT  $\checkmark$  610 mM DKT  $\checkmark$  610 mM DKT  $\checkmark$  610 mM DKT  $\checkmark$  610 mM DKT  $\checkmark$  610 mM DKT  $\checkmark$  610 mM DKT  $\checkmark$  610 mM DKT  $\checkmark$  610 mM DKT  $\checkmark$  610 mM DKT  $\checkmark$  610 mM DKT  $\checkmark$  6

Witnessed &amp; Understood by me,

DeeAnna Polarp

Date

2/16/95

Invented by

Recorded by

Date

2-1-95

To Page No.

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

1/6/95

155

6.4 kb

ag No. \_\_\_\_\_

Purpose: To repeat & optimize preliminarily 6.4 kb.

Tried mini-prep done as a control

Will try 2 dif. cycling conditions 3 step as well as 2 step.

Colonies will be lysed in 2 different ways 1. in PK (single)  
2. in H<sub>2</sub>O colony  
unlyzed will also be included again. Bufferconditions: - since 200 µl dNTP + 2 mM pH 7.5, 10 mM Tris-HCl  
4 µl per primer has Mg 1 mM EDTA  
worked with Tag 5U, the same 50 µg/ml PK  
conditions will be used. ↓used 2 µl of mini-prep - can unknown (used BMB (3).  
still have to run gel)Tried dif. enzyme conc 1, 2, 5 and 1:0.01, 2:0.02, 5:0.05  
Tag Tag & 2VColony lysis: Since these colonies were so minute after or  
at 37° pooled 5 or 6 colonies in a single area -  
spotted 2 µl of lysis buffer or H<sub>2</sub>O mixed & pipetted out the  
liquid onto a tube containing 10 µl of lysis buffer  
or H<sub>2</sub>O

Colonies in PK lysis 55°, 15' → 95°, 15'

in H<sub>2</sub>O 95°, 15'(Added ~ 5 µl of H<sub>2</sub>O) pooled all three tubes together  
and made up the volume to 50 µlShould have picked more for more reactions  
used 10 µl / Rx - appropriately either PK lysed or H<sub>2</sub>O lysed or  
colony itself 10 µl H<sub>2</sub>O To Page N

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Date

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K. Subraman

1/6/95

From Page No. \_\_\_\_\_

For mini-prep DNA: prepared premix with template

For colony: added them later

mini-prep premix: 25x

A

dNTP 25  $\mu$ l (200  $\mu$ M each / Rx)P.P. 5 (25  $\mu$ l) 0.4  $\mu$ MA.P. 5 0.4  $\mu$ MTemplate 25x2  $\rightarrow$  last exp. used 1.5 + 1 / Rxmini-prep = 2  $\mu$ l / RxH<sub>2</sub>O 415

500

 $\rightarrow$  20  $\mu$ l / Rxpremix B: 5xTag

1

2

5

Tag + DV

1:01

2:02

5:00

(2  $\mu$ M) Buffer B

50

(100x) enzyme

5

1

2.5

5

10

25

H<sub>2</sub>O

99.5

99

97.5

95

90

75

150

 $\rightarrow$  30  $\mu$ l / Rxstep 2  
3 cycle2 step 1

94°, 3'

2x (94°, 45"

55°, 30"

72°, 3')

94°, 3'

94°, 45"

68°, 5'

20

T Page 1

Witnessed &amp; Understood by m,

Date

Invented by

Date

Recorded by

Dr. S. S. Suman

1/6/94

| age No. _____ |  | 2 step  | 3 step |
|---------------|--|---------|--------|
| Tube #        |  |         |        |
| 1             |  | 1       | 13     |
| 2             |  | 1       | 14     |
| 3             |  | 2       | 15     |
| 4             |  | 2       | 16     |
| 5             |  | 5       | 17     |
| 6             |  | 5       | 18     |
| 7             |  | 1 : .01 | 19     |
| 8             |  | 1 : .01 | 20     |
| 9             |  | 2 : .02 | 21     |
| 10            |  | 2 : .02 | 22     |
| 11            |  | 5 : .05 | 23     |
| 12            |  | 5 : .05 | 24     |

Colonies

The mix A : 15 x

Mix B : 5 x as earlier :  
for T + D.V

dntp 15

primer 3

" 3

- 150 (template 10 µl / Rx (the added later))

L20 129

150 → 10 µl f Rx

20 µl → + ←

added 30 µl / Rx

appropriately either

Tag alone or Tag + Rx

changing condition same as mini prep.

for 3 step cycle, 2 step not done. " didn't

have much template left from  
lysed plasma

tube # 25 1 + .01

26 2 + .02

27 5 + .05

PK lysed

28 } 11,20

29 } lysed

30 }

31 }

32 }

33 }

straight piece

.35, 36 (20 Tag)

Hw plain.

T Pag No. \_\_\_\_\_

is d &amp; Understood by m ,

Date

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Dat

Rec rded by

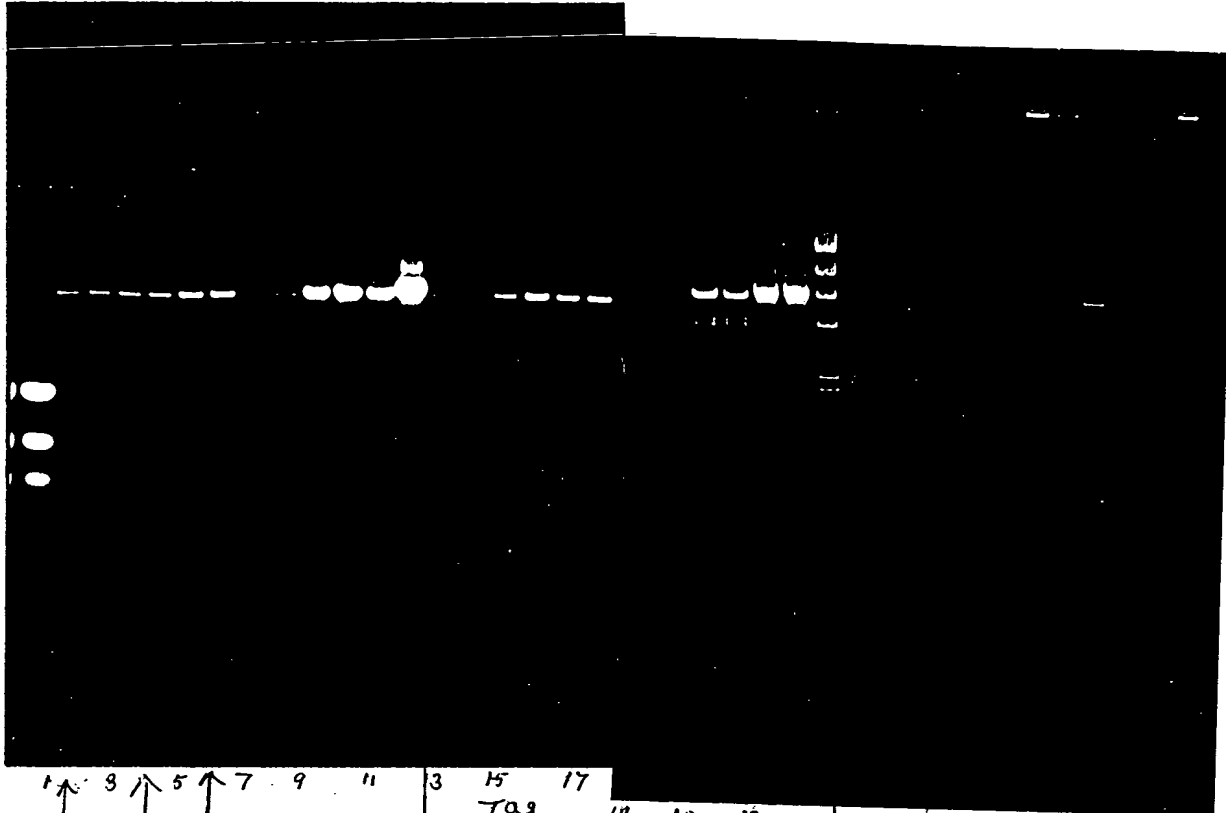
1/6/94

K. Sitarman

From Page No. \_\_\_\_\_

2 step cycle

3 step cycle



1 3 5 7 9 11 13 15 17  
Tag

19 21 23  
T + DV

pk direct  
H2O pack

Unit 1  
enzyme  
Tag + DV  
1 2 5  
Fingerprint  
Mix  
1, 2, 5

pk H2O direct  
typed typed  
w. Tag + DV  
w. Tag + DV  
2 U

mini prep.

plasmids

Result: Even 3 step gave better product with less mis-  
plasmid amp should be done under more control.

To Page 1

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Dat

1/20/95

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K. Stareman

Date

1/19/95

Continued from P 111

SSI and Old I cuts of materials

**Project N** .\_\_\_\_\_

**Book No.**\_\_\_\_\_

age N — *run at 1 hr* *post test control* *control*

↓ ↓

up # (1) 1 2 3 4 5 6 7 8 9 10 (11) 12 13 (14) 15 16 17 17 18 20 (21)

30 54 57 64 73 87 92 105 118 113 120 30 3 29 30 20 39 71 74 75 76 31

10 10  $\mu$ l —————→ 10 —————→ 25.6 —————→

buffer 4 2 2 —————→ 2 —————→ 3 —————→ ✓

I 1 1 —————→

✓ II ✓ 0.3 —————→ 0.45 —————→ ✓

✓ II ✓ 0.1 —————→ 0.15 —————→ ✓

✓ I 10.5 —————→

- R I 0.75 —————→ ✓

7

7 —————→ 12.1 —————→

$V_p = 20 \mu$ l  $V_p = 20 \mu$ l 30  $\mu$ l

37°C 2 hr

was only  $\sim 1/2$  wt by 1 hr  
 did 0.5 ml more ~~very~~

for DrdI

|           |           |
|-----------|-----------|
| mini prep | 6p        |
| #         | Proquents |

control 2.7kb (mut), 1.8, 0.8 full length  
#73 " " " " Caeprax

58.54  
87.120

54, 72, 103  
113

1.8

## Results

full Length  
Cue present

miniprep  
3 and 24  
have full  
length loc  
bases on primers  
of 410, 465 bp  
30 P1 was probably  
small or point  
mutation

all <sup>the</sup> not  
results  
of P.I. 1  
all full  
finger  
less based  
on pictures  
of 410 465 bp  
chain more OK  
cut here

↑  
mine  
#75  
has no bps  
full length  
low in  
percent

**To Page No.\_\_\_\_**

ed & Understood by m ,

Lucia Polanco

**Date**

2/16/95

Inv nt d by

**Recorded by**

Date \_\_\_\_\_

225

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

From Page No. \_\_\_\_\_

| <u>miniprep #</u> | <u>Mn (mm)</u> | <u>Deep Vent</u> | <u>Pull length<br/>lac</u> | <u>percent<br/>rearrangements</u> |
|-------------------|----------------|------------------|----------------------------|-----------------------------------|
| 1-20              | 0              |                  | 19                         | 5%                                |
| 21-40             | .05            |                  | 20                         | 0                                 |
| 41-60             | 0.1            |                  | 18                         | 10%                               |
| 61-80             | 0              | +                | 17                         | 15%                               |
| 81-100            | .05            | +                | 18                         | 10%                               |
| 101-120           | 0.1            | +                | 16                         | 20%                               |

To Page 1

Witnessed &amp; Understood by me,

Deena Polay

Date

2/16/95

Invent d by

Record d by

Date

10.5 kb

ig No. \_\_\_\_\_

10/9/95: To try another miniprep - 10.5 kb fragment contained in pDELTA1 - and amplify

used overnight cultures grown in the presence of Tetr + Kan  
saved at 4°, over wk end.

Alkaline lysis protocol. all resuspended in 25 µl of TE  
extracted from 3 x 1.5 ml culture

Did an enzyme titration } - amplification done only with  
Mg " } miniprep DNA. no plasmids  
were used. gels.

included was just 1 rx with Tag & 20

Vol 50 µl. 200 µM dNTP  
4, 3, 2 mM Mg  
4 mM primer  
{ 1, 2, 5 U enzyme }  
0.1 0.1 0.5

cycling 3 step 95°, 3'  
(95°, 45"  
55°, 30"  
72°, 5") 25 cycles

prepared master with Buffer B containing 2 mM Mg

added supplement Mg accordingly.

| 2 mM | 3 mM | 4 mM |
|------|------|------|
| 0    | 5    | 10   |
| 50   | 45   | 40   |
| 50   | 50   | 50   |

add 2 µl of miniprep template  
con. unknown.

prepared 20x mix

|          |     |           |    |
|----------|-----|-----------|----|
| x buffer | 100 | } 40 x 20 | 40 |
| dNTP     | 20  |           | 5  |
| P1       | 4   |           | 5  |
| P2       | 4   |           | 5  |
| temp.    | 40  |           | 50 |
| enzyme   | -   | (in 5 µl) |    |
| Mg       | -   | ( " )     |    |

5 µl / rx as needed.  
enzyme 1 µl added  
5 + 2 - 17 later  
def. con.

To Page No. \_\_\_\_\_

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Date

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Recorded by

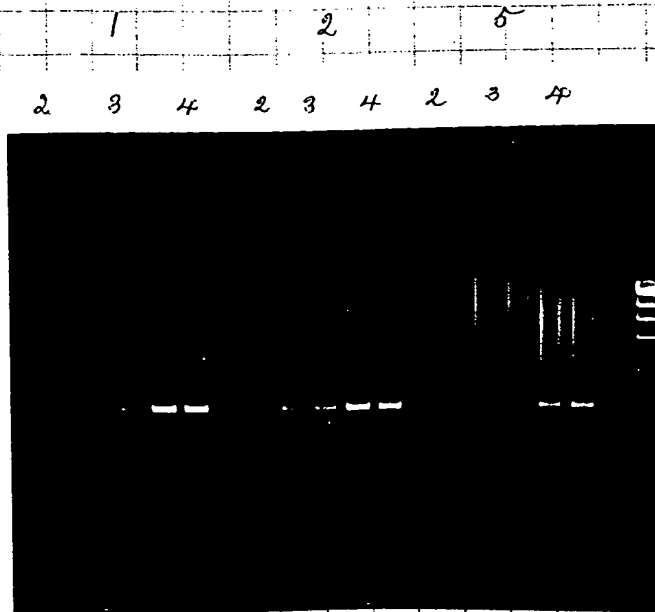
1/10/95

K. S. Karan

From Page No. \_\_\_\_\_

|    |    |    |   |
|----|----|----|---|
| 1  | 2  |    | 2 |
| 3  | 4  | 10 | 3 |
| 5  | 6  |    | 4 |
| 7  | 8  |    | 2 |
| 9  | 10 | 20 | 3 |
| 11 | 12 |    | 4 |
| 13 | 14 |    | 2 |
| 15 | 16 | 50 | 3 |
| 17 | 18 |    | 4 |

19 Tag 20 2 ml

Result :

cycling has to be optimized. - Lot of mispriming  
2 mM Mg didn't work in any of the sets? - It  
worked earlier in 25 µl as w Tag.

- amount of template?

- Increasing the enzyme didn't seem to work.  
as does Mg

- Tag alone at 20 / 50 µl didn't work

- get fresh enzyme.

Witnessed &amp; Understood by m ,

Dat

1/25/85

Inv nted by

R corded by

Dr. Subraman.

Date

1/10/84

To Pag

# Stability study for PCR mix containing 1 unit of Tag

Exhibit 37  
Appl. No. 09/558,421

B K N .

Assay date is  
2-3-95 121

| Page No. _____              | al enzyme added to reaction | Tag with or without A22 | * put 3ul H <sub>2</sub> O/4 mix 10 1X = 48ul mix so + 2 X Enz gives 1/2 = 50 |
|-----------------------------|-----------------------------|-------------------------|---|
| 1 experiments               |                             |                         |   |
| J. Soler of 1-20-95         | Rxn #                       |                         |   |
| 2x R26 0.1% TN (Tween/NP40) | 1-3                         | 2 (0.05%) Tween 20/NP40 |   |
| 0.2% BS Brij                | 4-6                         |                         |   |
| 0.2% TX Triton              | 7-9                         |                         |   |
| 0.01% TN                    | 10-12                       | ← (.0004%)              |   |
| 0.02% BS                    | 13-15                       |                         |   |
| .02% TX                     | 16-18                       |                         |   |
| 1.0% TN                     | 19-21                       | (.04%)                  |   |
| 2.0 BS Brij                 | 22-24                       |                         |   |
| 2.0 TX                      | 25-27                       |                         |   |
| No detergent                | 28-30                       |                         |   |
| (1.1X)                      | 31-33                       | 3.64                    |   |
| (5X) → dilute 1/2.5 = .04%  | 34-36                       | 2                       |   |
| 2x R26 @ 0.1% + Enz         | 37-39                       |                         |   |
| 2x TFI 0.1%                 | 40-42                       |                         |   |
| 2x Vent buffer              | 43-45                       |                         |   |
| 5ul                         |                             |                         |   |
| dil = 0.04%/ul              | 46-50                       | 2                       |   |

Reassay on 10  
this page 2-3-95  
152 3-9-95  
167 4-4-95  
36, 10 5-26-95  
52, 10 5-27

10' 74°C, 10ul 10' 74°C  
spot 40ul on 6 FC  
dilution used 10ul dil buffer of 9-20-94 (see new stocks)

| Test Rxn mix         | Mix P. 120 |
|----------------------|------------|
| EKBT 15%/ul 1-31-95: |            |
| no dil               | 2 46ul     |
| 1/125                | 2          |
| EP9407               | 2          |
| 1/125                | 2          |

made new mix with stock shown in red on P120 and repeated experiment on 2-3-95 - results on next page (P122)

incorporation!

label 5ul of #12 (5X) into 12ul Tag dil buffer P55, 7

|  |                 |                 |                          |
|--|-----------------|-----------------|--------------------------|
| Read & Understood by me,<br>Wanda Polans | Date<br>2/10/95 | Invent d by<br> | Date<br>2-1-95<br>2-3-95 |
|  |                 | R corded by     |                          |

To Page No. \_\_\_\_\_

122

Project No. \_\_\_\_\_

Book No. Av2TITLE unit/ptRelative  
to Tray

Tray = 5

u/x N  
to Tray

From Pr ...

|      |    |          |   |      |        |  |               |
|------|----|----------|---|------|--------|--|---------------|
| 1E   | 1  | 8410.00  | } | 8819 | .037   |  | .03           |
|      | 2  | 9136.00  |   |      |        |  |               |
|      | 3  | 8912.00  |   |      |        |  |               |
|      | 4  | 7465.00  | } | 7552 | .033   |  | .03           |
| 2E   | 5  | 8664.00  |   |      |        |  |               |
|      | 6  | 7728.00  |   |      |        |  |               |
|      | 7  | 7737.00  | } | 7580 | .032   |  | .03           |
| 3E   | 8  | 7235.00  |   |      |        |  |               |
|      | 9  | 7769.00  |   |      |        |  |               |
|      | 10 | 7579.00  | } | 6878 | .029   | ✓  | .02           |
| 4E   | 11 | (3001)00 |   |      |        |  |               |
|      | 12 | 6178.00  |   |      |        |  |               |
|      | 13 | 7484.00  | } | 7812 | .033   |  | .03           |
| 5E   | 14 | 7833.00  |   |      |        |  |               |
|      | 15 | 8119.00  |   |      |        |  |               |
|      | 16 | 6228.00  | } | 6566 | .027   | ✓  | .02           |
| 6E   | 17 | 6715.00  |   |      |        |  |               |
|      | 18 | 6755.00  |   |      |        |  |               |
|      | 19 | 8215.00  | } | 7824 | .033   |  | .03           |
| 7E   | 20 | 8743.00  |   |      |        |  |               |
|      | 21 | 6514.00  |   |      |        |  |               |
|      | 22 | 7996.00  | } | 8413 | .035   |  | .03           |
| 8E   | 23 | 8661.00  |   |      |        |  |               |
|      | 24 | 8581.00  |   |      |        |  |               |
|      | 25 | 7644.00  | } | 7533 | .031   |  | .03           |
| 9E   | 26 | 6981.00  |   |      |        |  |               |
|      | 27 | 7976.00  |   |      |        |  |               |
|      | 28 | 4900.00  | } | 4989 | .021   | } no detergent looks low. Can try<br>+ detergent in unit assay | .02           |
| 10E  | 29 | 4647.00  |   |      |        |  |               |
|      | 30 | 5419.00  |   |      |        |  |               |
|      | 31 | 7509.00  | } | 7702 | .032   |  | .03           |
| 11E  | 32 | 6923.00  |   |      |        |  |               |
|      | 33 | 8674.00  |   |      |        |  |               |
|      | 34 | 8196.00  | } | 8075 | .034   |  | .03           |
| 12E  | 35 | 7970.00  |   |      |        |  |               |
|      | 36 | 8060.00  |   |      |        |  |               |
|      | 37 | 8015.00  | } | 7442 | .031   |  | .03           |
| 3E   | 38 | 7358.00  |   |      |        |  |               |
|      | 39 | 6954.00  |   |      |        |  |               |
|      | 40 | 8055.00  | } | 8479 | .035   |  | .03           |
| 4E   | 41 | 8359.00  |   |      |        |  |               |
|      | 42 | 9023.00  |   |      |        |  |               |
|      | 43 | 7844.00  | } | 7611 | .032   |  | .03           |
| 5E   | 44 | 7351.00  |   |      |        |  |               |
|      | 45 | 7638.00  |   |      |        |  |               |
|      | 46 | 9312.00  | } | 9580 | (0.04) |  | .04           |
|      | 47 | 9496.00  |   |      |        |  |               |
|      | 48 | 9290.00  |   |      |        |  |               |
|      | 49 | 9726.00  | } |      |        |  | by Definitive |
|      | 50 | 10073.00 |   |      |        |  |               |
|      | 51 | 58661.00 |   |      |        |  |               |
| ativ | 52 | 60427.00 | } |      |        |  |               |
|      |    |          |   |      |        |  |               |

ave = 59544  $\Rightarrow$  1,478,600 cpm/50  $\times$  12  $\times$  2

37.2 cpm/pmol

To Page No

Witnessed &amp; Understood by me,

Deena a Polanco

Date

2/16/95

Invented by

Rec rd by

Date

2-3-95

11/9/95

161

plates / 6.4, 8, 10.5, 20, 29 kb.

Page No. \_\_\_\_\_

Purpose: ~~to~~ make more cleaner plates of 6.4, 8, 10.5, 20, 29 kb.

plated quite a few for each at diff. conditions

these were grown under stringer conditions in the presence of Tel + Kan.

Read Amp plates, left at 37° overnight.

6.4, 8.0 - colonies were small, especially 6.4

same each time whenever tried. - don't remember how they were last time

Rest looked ok. - well spread out bigger colonies.

streak them all at 4°.

picked a few single colonies from 6.4 & 8.0  
grew them again for fresh minipreps. grown under Kan + Tel conditions.

from 8.0 - did minipreps by alkaline lysis method, resuspended in 15 µl 95% x12 = 180 µl Total.

6.4 kb streak at 4°.

To Page No. \_\_\_\_\_

Read &amp; Understood by me,

Date

Invented by

Recorded by

Date

11/11/95

Project No. \_\_\_\_\_  
Book No. \_\_\_\_\_

(see P 80)  
TITLE  $^{32}P$  23mer depuration reaction conditions

126

From Page No. tube # 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22

SX mixes

P. 125

$\leftarrow$  (0.65 pmol/ $\mu$ l)  
 $^{32}P$  23mer 0.267 pmol primer  
(mixed as P. 75)

Vent pol  
0.01 u/ $\mu$ l

Whitish in Vent storage  
no dilution buffer  
from NEB

50% glycerol

H<sub>2</sub>O

$V_f = 20$

add 10  $\mu$ l cycle seq stop

(13 Rxns)

cocktail

[A]

[B]

$^{32}P$  23

H<sub>2</sub>O

50% glycerol

$V_f = 182$

add H<sub>2</sub>O/tube # 1-10

and 23 which  
gets 2  $\mu$ l of  
Vent dil buffer

7.8  $\lambda$

174.2

7.8  $\lambda$

137.8

36.4

182

# 11-20

preheat tubes to 70°C,  
then add Vent  
for 30 min 12 min

20  $\mu$ l Rxn has 0.16 pmol primer = 18 nM primer

0.01 unit Vent at 100,000 u/mg  $\approx$

MW  $\approx$  100,000

$\Rightarrow \approx 0.1$  pmol pol / 1 unit  
= (0.002 pmol pol total pol)

0.16 pmol primer

0.002 pmol pol

= 80 primer/pol

0.38 pmol circles / 0.002 pmol pol = 192 circle/pol

\*  $^{32}P$  23-mp 19

OH 7.5

1.1M Tris

0.1  $\mu$ l .3

$^{32}P$  23

(0.267 pmol primer/ $\lambda$ ) P. 75

0.6  $\mu$ l 1.8

0.47 pmol primer

113 m p 18 + 0.2  $\mu$ g/ $\lambda$

= 0.084 pmol circles/ $\mu$ l

9.3 27.9

2.5 2.78 pmol circles

17  $\mu$ l

30

70°C, 5' cool down

To Page No

Witnessed & Understood by m,  
Dennis R. Boland

Date

2

Invented by

Rec rd by

Dat

2-10-95

1/5 but dil buff  
1/5 but dil buff  
CF  
50 mM Tris HEPH 7.4  
1 mM DTT  
0.10% NP40, Tween 20 each  
50% glycerol  
100 mM K-Cl  
Run 8% gel with poor resolution,  
run 16% PAGE plus new reactions on 2-13-95

16% PAGE see P 144, 1

|                | # 24                        | 25   | 26   | 27   | 28  | 29  | 30   | 31   | 32  | 33  |
|----------------|-----------------------------|------|------|------|-----|-----|------|------|-----|-----|
| antibuffer     | 2                           | 2    |      |      |     |     |      |      | 2   | ✓   |
| 1, P125        |                             |      |      |      |     |     |      |      |     |     |
| antibuffer     |                             |      | 4    | 4    |     |     |      |      |     | ✓   |
| avg PPO = 83   |                             |      |      |      | 4   | 4   |      |      | 4   | ✓   |
| 20 mM          |                             |      |      |      | 2   | 2   |      |      | 2   | ✓   |
| #10 P125       |                             |      |      |      |     |     | 4    | 4    |     | ✓   |
| Cheng mix      |                             |      |      |      |     |     |      |      |     | ✓   |
| or (P126)      | 0.6                         |      |      |      |     |     |      |      |     | ✓   |
| 19 (P126)      | 0.077 pmol circ / $\lambda$ |      |      |      |     |     |      |      | 5   | 5   |
| 0.01 $\mu$ A   | 2                           | 2    |      |      | 2   |     | 2    |      |     | ✓   |
| 0.1 $\mu$ A    |                             | 2    |      | 2    |     | 2   |      | 2    | 2   | ✓   |
| glycerol       |                             |      |      |      | 2.8 |     |      |      | 2.8 | ✓   |
|                | 15.4                        | 15.4 | 13.4 | 13.4 | 8.6 | 8.6 | 13.4 | 13.4 | 11  | 4.2 |
| $V_f = 20 \mu$ |                             |      |      |      |     |     |      |      |     | ✓   |

my Cheng has on 1% glycerol at 1X need 7% more

40% Acrylamide 200g  
0.8% Bis 4g  
H<sub>2</sub>O

70°C, 12'  
PAGE  
start 1700 V at 1:45 pm  
at 25 V/10, 15 mAmp  
1-10 11-20 pri 21, 22 24-32 pri 10 empty  
space space space big plate

get ~ 7.4 cm/hr  
need 3 hr

500 ml  
went to 30 with constant set 2200-2250 V  
8.7 cm/hr

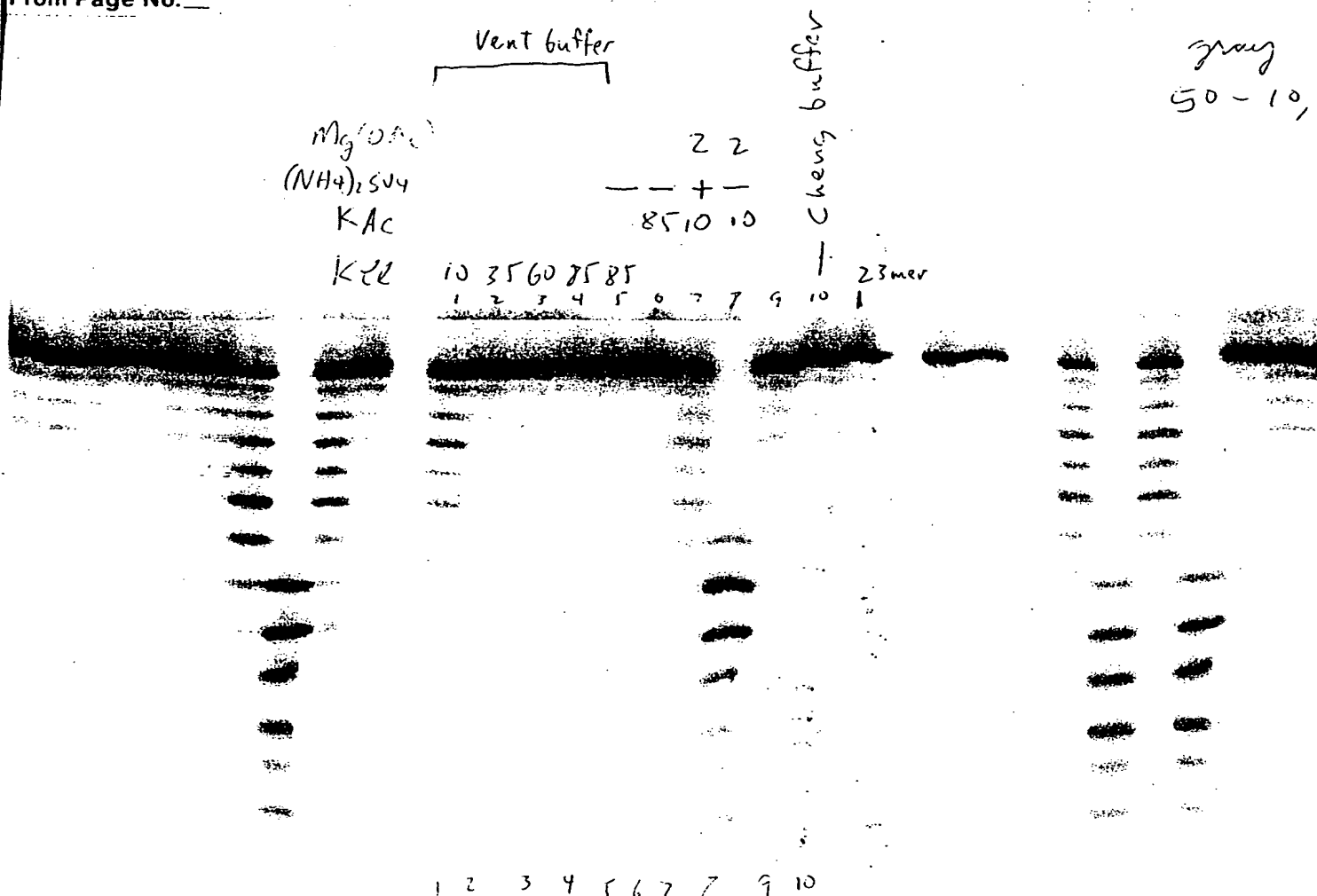
128

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

From Page No. \_\_\_\_\_



# Results:

- #1, 10 KCl +  $MgSO_4$  is same as KOAc,  $MgOAc$  - get degradation if  $K \leq 1$
- 1-5 increasing ionic strength eliminates degradation. #5 also 85 mM KAc same as KCl 85 mM
- 8 leave out  $(NH_4)_2SO_4$  get best result degradation of all (don't have (-)  $(NH_4)_2SO_4$  for 10 mM KCl and  $MgSO_4$  only 85 mM this result also consistent with ionic strength effect
- 9 substitute tricine for Tris in Vent buffer has no effect
- 10 complete Cheng buffer - no degradation can be fully explained as due to 85 mM KAc - see # 4, 5 - 85 mM KCl or KAc no degradation in Vent buffer

Witnessed & Understood by m ,

Date

Invented by

Date

T Pag

2/16/95

Record d by

2-13-95

- 02/14/95 - 06:45 pm

1.00x Counts

49.97



10000.00

D

Vent buffer  
 NEB RL 5x G-TM RL  
 .02 .2 .02 .2 .02 .2 .02 .2  
 Cheng  
 Vent (NEB) 23-mp/19  
 Cheng G-TM 23-mp/19  
 23 mer  
 :units  
 Vent

23 mer is strongly  
 protected when annealed.  
 to m13 ssDNA

To Page No.

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Date

2/16/95

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Date

Recorded by

suave a Polars

130 The vs Toy Project No. \_\_\_\_\_ Book No. \_\_\_\_\_ TITLE effect of Kcl on pol on M17 and primer degradation

From Page No. \_\_\_\_\_

|   | (1)         | (2)         | (3)         | (4)         | (5)         | (6)         |                 |
|---|-------------|-------------|-------------|-------------|-------------|-------------|-----------------|
| Kcl mM  | 50          | 75          | 100         | 50          | 75          | 100         |                 |
| 10 X Tag PCR buff   | 8 $\mu$ l   | 8 $\mu$ l   | 8 $\mu$ l   | 8 $\mu$ l   | 8 $\mu$ l   | 8 $\mu$ l   | ✓ ✓             |
| Kcl 0.5M  | 4           | 8           |             | 4           | 8           |             | ✓ ✓             |
| $^{32}P$ 23. mer (0.064 pmol $\frac{23. \text{mer}}{\lambda}$ ) | 8 $\mu$ l   | 8 $\mu$ l   | 8 $\mu$ l   | 8 $\mu$ l   | 8 $\mu$ l   | 8 $\mu$ l   | ✓ ✓ (0.064 pmol |
| 10 mM 4dNTPs  | 1.6 $\mu$ l | 1.6 $\mu$ l | 1.6 $\mu$ l | 1.6 $\mu$ l | 1.6 $\mu$ l | 1.6 $\mu$ l | ✓ ✓ 200         |
| MgCl <sub>2</sub> 50 mM   | 2.4 $\mu$ l | 2.4 $\mu$ l | 2.4 $\mu$ l | 2.4 $\mu$ l | 2.4 $\mu$ l | 2.4 $\mu$ l | ✓ ✓ 1.5         |
| Tag 0.4 $\mu$ /l  | 2           | 2           | 2           | 2           | 2           | 2           | 0.8             |
| Tne 0.8 $\mu$ /l  |             |             |             | 2           | 2           | 2           | 1.6             |
| H <sub>2</sub> O  | 58          | 54          | 50          | 58          | 54          | 50          | ✓ ✓ 0.2 $\mu$   |
| preheat tube to 70°C, start with 2 $\mu$ l pol                  |             |             |             |             |             |             | VP = 10 $\mu$ l |

remove 10  $\mu$ l at 1, 2, 5, 10 min to 5  $\mu$ l cycle seq stop

\* rTag EKBT1 1-31-95 5  $\mu$ /l } both diluted in Tag dil buffer  
Tne 5  $\mu$ /l A. Golden

$^{32}P$  23 mer same as P.75 (0.267 pmol 23 mer / l)

$^{32}P$  23. mer

|                  |  |              |   |
|------------------|--|--------------|---|
| $^{32}P$ 23. mer | 0.267 pmol 23 mer / l                      | 15.8 $\mu$ l | 14.2 pmol 23 mer tr                         |
| M13 mp19         | 0.2 $\mu$ g / l<br>(0.084 pmol circle / l) | 50 $\mu$ l   | (4.2 pmol circle                            |
| 1, mTas 7.5      | 0.6  | 66 $\mu$ l   | 0.064 pmol $\frac{23. \text{mer}}{\lambda}$ |
|                  |  |              | use 1 $\mu$ l / 10 $\mu$ l re               |

To Page 1

Witness d & Und rstood by m ,

Deena aBslamp

Date

2/16/95

Inv nted by

Re ord d by

Date

2-15-95

# Primer degradation (see P80)

Project No. \_\_\_\_\_

Block No. \_\_\_\_\_

131

| ag N           | 25-27 | 28-30 | 31-33 | 34-36 | 37-39 | 40   |   |
|----------------|-------|-------|-------|-------|-------|------|---|
| vent           | 8     | 8     | 8     | 8     | 8     | 8    | Does DMSO have any effect on contaminant?       |
| Tag PCR buffer |       |       | 8     | 8     | 8     |      | ✓   |
| DMSO           |       | 1.6   |       |       |       | 1.6  | ✓ Cf = 2% DMSO                                  |
| 23 min         | 1.91  |       |       |       |       |      | ✓ (-0.64 pmol 23 min / 10 μl) (= 6.4 μM primer) |
| 1/2 5 min      | -     | -     | 2.4   |       |       |      | ✓ (note Vent buffer has 2 min MgS)              |
| 5 min KCl      |       |       | 4     | 8     |       |      | ✓   |
| 0.7%           | 2     |       |       |       |       |      |   |
| 0              | 6.6   | 64.4  | 69    | 63.7  | 59.7  | 55.7 | 66.5 ✓  |

heat to 70°C, remove 10 μl at 2, 5, 15 min only, take 40

pol/circles

0.1 unit Tag = 0.005 pmol (per 10 μl Rxn)

0.064 pmol 23 min / 10 μl (= 0.464 pmol at 10 μl)

pmol circle / pmol pol 0.012 3 ends / pol molecules

Expected units

0.1 u Tag gives 1 nmol at 30'

have 0.464 nmol at 23 min / 10 μl reaction volume

so need ~14 min to replicate all DNA at least based on units - (but not sure M13 gives same units)

1 min would be ≤ 500 at extension at unit value rate

compared to PCR

Tag/time

1. This would be 0.5 units / 5 μl PCR

2. 6.4 nM primers (so 10 x less than 10 nM primers.)

T Page No. \_\_\_\_\_

Used & Understood by me,

Veronica Polansky

Date

2/16/95

Invented by

Recorded by

Date

2-15-95

132

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

From Page No. \_\_\_\_\_

100 bp ladder cut 10072-015

10  $\mu$ l H<sub>2</sub>O (vortex)

1  $\mu$ l 10 mCi/ml <sup>32</sup>P dCTP

15' 37°C  $\rightarrow$  10 $\times$  0.2 m EDTA

get total  $> 10^7$  cpm

load 0.2  $\mu$ l

$\frac{(20 \times \text{total})}{20 \text{ brands}}$

$10^7 \text{ cpm} \rightarrow 5000 \text{ cpm/brand}$

after 10  $\mu$ l EDTA

put 20  $\mu$ l (Rxn + EDTA)  
10  $\mu$ l counts seq stop

30  $\mu$ l  $\geq$  300,000 cpm  $\mu$ l  
10,000 cpm/brand/ $\mu$ l

30 load 1  $\mu$ l

To Page N

Witnessed & Understood by me,

*Deena a Pokar*

Date

3/16/95

Invent d by

*[Signature]*

Dat

2-18-95

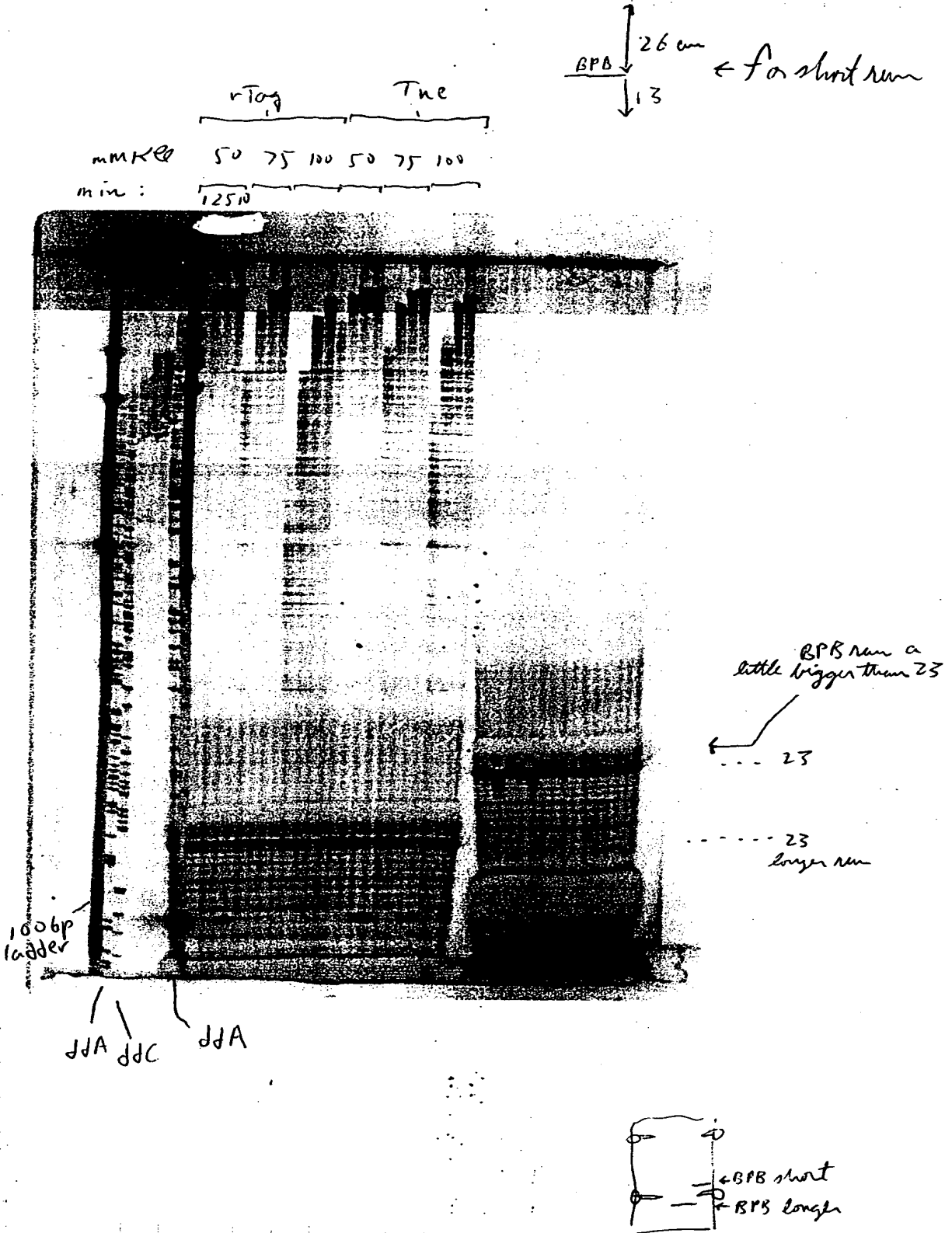
Record d by

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

From Page No. \_\_\_\_\_



To Page N

Witnessed & Understood by me,

Date

Invent d by

Date

*Deena Adkins*

3/16/95

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2-17-95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_ TITLE \_\_\_\_\_

136

From Page No. \_\_\_\_\_

primer 560826 (23 mer with terminal A instead of G called "AC")

74.6 n mol total  
746 x H<sub>2</sub>O

Cp = 100 pmol primer /  $\mu$ l (= 100  $\mu$ M prim)

Kinase

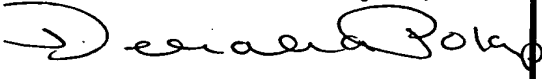
|   |                             |                           |            |   |   |              |
|---|-----------------------------|---------------------------|------------|---|---|--------------|
| 23 mer "AC"                                   | (100 $\mu$ M primer)        | 100 pmol 23 mer / $\mu$ l | 2 $\mu$ l  | ✓ | ✓ | 200 p        |
| 5x Kinase buffer                              |                             |                           | 8          | ✓ | ✓ | 23 ~         |
| <sup>32</sup> P $\gamma$ ATP 10 mCi / $\mu$ l | (3.3 $\mu$ M ATP)           |                           | 20         |   | ✓ | 66 p         |
| PNK 1 u / $\mu$ l                             |                             |                           | 2          |   |   |              |
| H <sub>2</sub> O                              |                             |                           | 7          | ✓ | ✓ |              |
| 30'   | 37°C $\Rightarrow$ 60°C, 5' |                           | 40 $\mu$ l |   |   | Cp = 5 $\mu$ |

use 2  $\mu$ l / 50  $\mu$ l PCR  
for 200 nM primer

note 1 unit T4 Kinase converts 1 n mol ATP / 30' at 37

To Page N

Witnessed & Understood by me,

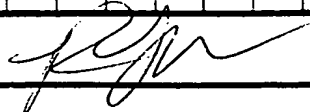


Date

3/16/95

Invented by

Rec rded by



Date

2-20-95

Primer degradation and under PCR conditions (200 nM primers 2 u pol  
and  $\Delta$  Kell

Pr | ct N

Bo k N

Exhibit 45

Appl. No. 09/558,421

137

| ge N                | 1           | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   | 11   | 12   |   |                   |
|---------------------|-------------|------|------|------|------|------|------|------|------|------|------|------|---|-------------------|
| XTag PCR buff       | 5A          |      |      |      |      |      |      |      |      |      |      |      | ✓ |                   |
| "AC" P136 (5 uM)    | 2A          |      |      |      |      |      |      |      |      |      |      |      | ✓ | (cf = 0.2 uM 230) |
| 20 0.5 M            | -           | 1    | 2    | 3    | 4    | 5    | -    | 1    | 2    | 3    | 4    | 5    | / |                   |
| ne 5 u/l →          |             |      |      |      |      |      |      |      |      |      |      |      |   |                   |
| dilute to 2 u/l     | 2 u/l       |      |      |      |      |      |      |      |      |      |      |      |   | (4 units total)   |
| 7.5 u/l 20 u/l      |             |      |      |      |      |      |      |      |      |      |      |      |   |                   |
| dilute to 0.2 u/l → |             |      |      |      |      |      |      |      |      |      |      |      |   | (0.4 units)       |
| neg 2 50 mM         | 10.5 A      |      |      |      |      |      |      |      |      |      |      |      | ✓ | (cf = 1.5 mM)     |
| H2O                 |             |      |      |      |      |      |      |      |      |      |      |      | ✓ |                   |
|                     | 39.532      | 34.5 | 34.5 | 34.5 | 34.5 | 34.5 | 34.5 | 34.5 | 34.5 | 34.5 | 34.5 | 34.5 |   |                   |
|                     | cf = 50 u/l |      |      |      |      |      |      |      |      |      |      |      |   |                   |

M M K cf = 50 60 70 70 90 100 50 60 70 70 90 100

70°C, remove 10 u/l to 5 u/l stop at 20, 60, 120 min

Results on P15T

To Page No. \_\_\_\_\_

ed & Understood by me,

Date

Inv nted by

Date

22/11/95

3/16/95

Recorded by

2-21-95

138

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE 33 miss matched and mismatched

From Page No. \_\_\_\_\_

11.64 nmol "33 correct"  
(primer # 5381 DG1 (G01))

582  $\mu$ l H<sub>2</sub>O

has correct G at 3' end at  
of P1 site in MCS G-3'  
GAATTC  
20  $\mu$ M primer

13.42 nmol  
33 mismatch

671  $\mu$ l H<sub>2</sub>O

20  $\mu$ M primer

33 correct 1  $\mu$ M  $\times$  5.3  $\checkmark$   $\checkmark$  (5.3 pmol total)  
(1 pmol/1)

33 mismatch 1  $\mu$ M 5.3  $\checkmark$   $\checkmark$   
(1 pmol/1)

Y ADP (1.62  $\mu$ M ADP)  $\times$  4 4  $\checkmark$   $\checkmark$   $\checkmark$  (6.68 pmol)  
5X Kinase buffer  $\times$  4 4  $\checkmark$   $\checkmark$   $\checkmark$

PNK 1  $\mu$ l 1  $\checkmark$   $\checkmark$   $\checkmark$   
H<sub>2</sub>O  $\times$  5.7 5.7  $\checkmark$   $\checkmark$

20  $\mu$ l 20  $\mu$ l 37°C, 30'  $\Rightarrow$  5'

1 pmol

2.108 pmol circle mp19 0.2  $\mu$ g/l  $\times$  36.7 36.7 0.3 pmol primer

1M Tris pH 7.5  $\times$  0.6 0.6  $\checkmark$  0.6 pmol circles

$\checkmark$   $\times$  20.7  $\checkmark$   
V<sub>2</sub> = 6.6 66  $\mu$ l circle/1  
5', 95°C cool slow = 2  
exce

use 2  $\times$  / 20  $\mu$ l reaction

= 0.6 pmol primer in 20  $\mu$ l

To Page 1

Witnessed & Understood by me,

*Demetri Polaris*

Date

3/16/95

Invented by

Recorded by

Date

2-23-95

GEL

- 02/24/95 - 01:10 pm

0.66x Counts

0.11

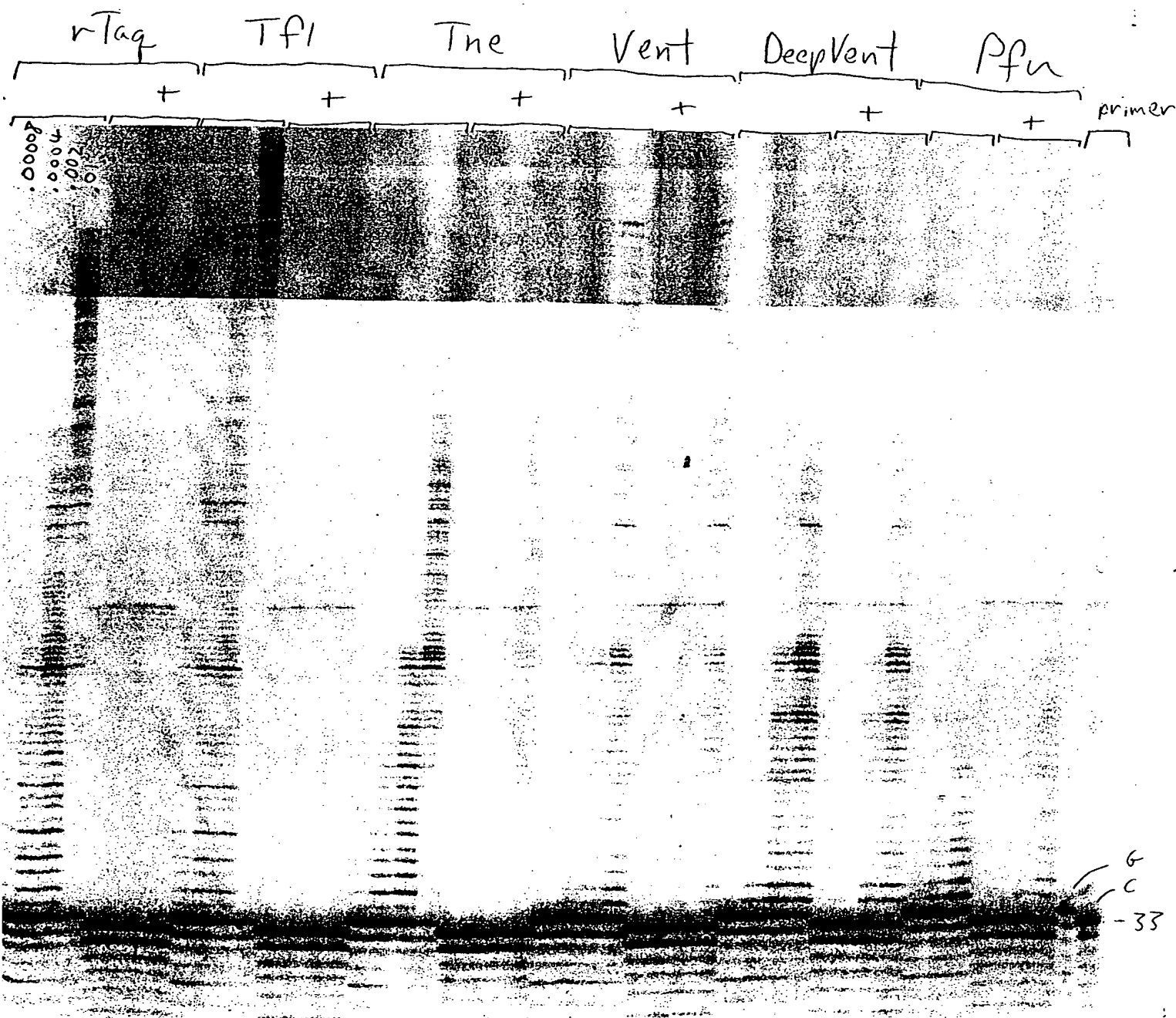


2000.25

D

Exhibit 47

Appl. No. 09/558,421



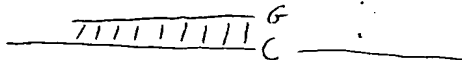
circles/pol molec

56

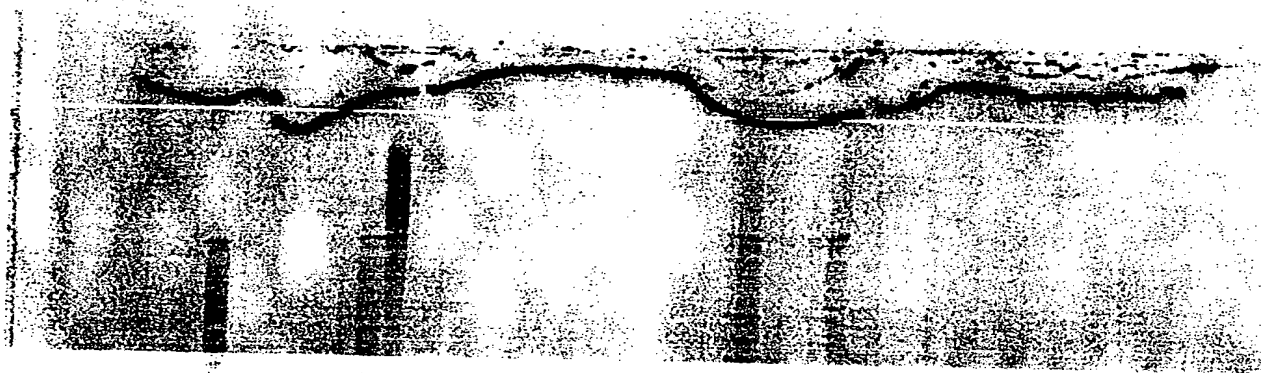
282

1410

7050



16% PAGE 33 watts 4 1/2 hr  
 XC went 21.7 cm ( $R_f = \frac{21.7}{39} = 0.56$ )  
 33 mer migrated 25 cm  
 XC runs as a 40 mer



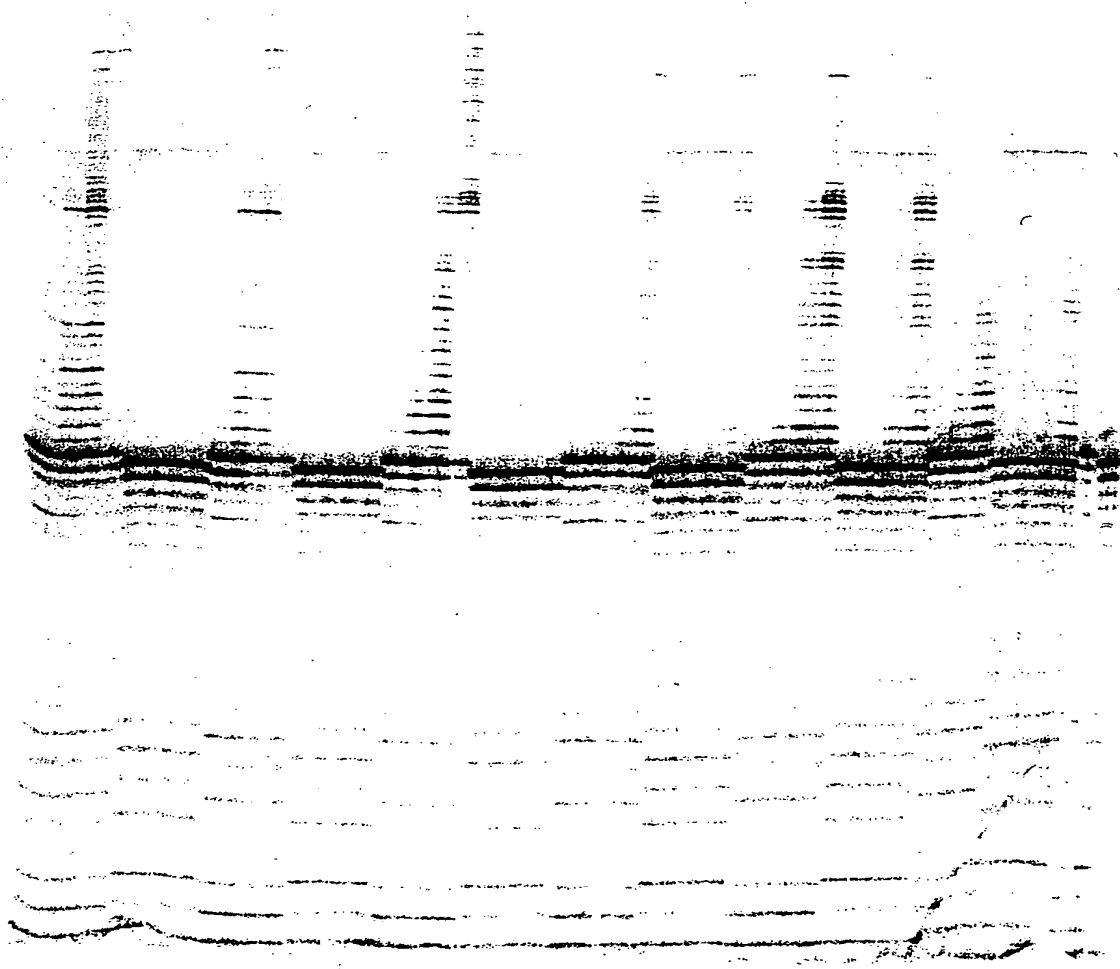
was  
 in as 39 mer)  
 (C →

3 →

XC →

33 mer →

16% →



could run XC to ~ 30 cm

ll. 33 mer went  
 25 cm of 39 mer  
 gel long the

To Page No. \_\_\_\_\_

**sed & Understood by me,**

Carla Polansky

Date \_\_\_\_\_

$$3 \overline{) 1695}$$

Inv nted by

Recorded by

Date \_\_\_\_\_

2-749

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

140

From Page No. \_\_\_\_\_

 $^{32}$ P correct. mp19 (P.138) $^{32}$ P mix. - mp19 (P.138)

10mM dNTPs each

50mM MgCl<sub>2</sub>

10x PCR buffer

10x Vent buffer

10x Pfu buffer

H<sub>2</sub>OrTag .00008  $\mu$ l

-31-ES .0004

.002

.01

Tfi .00008

Pfu .0004

130000A .002

.01

Tne .00008

.0004

.002

.01

Vent .00008

.0004

.002

.01

DeepVent .00008

.0004

.002

.01

Pfu .00008

.0004

.002

.01

To Page 1

Witnessed &amp; Understood by me,

Deena Bobay

Date

3/16/95

Invented by

Record d by

Date

2-24-95



Project No. \_\_\_\_\_  
Book No. \_\_\_\_\_

TITLE Repair of 3' mismatch  
for TFI  $\pm$  Vent and rTog  $\pm$  DV, Pfu Tr

142

| From Page No. —                                      | * | 1    | 2  | 3    | 4  | 5    | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|--|---|------|----|------|----|------|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|
| 32 P33 mis - m pl9<br>(P138 en. of 0.01 pm primer/1) |   | 2    |    |      |    |      |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 10 mM JNTPs  |   |      |    |      |    |      |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 5X Chngy complete (2/14/95)                          | 4 |      |    |      |    |      |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 5X elongase<br>H <sub>2</sub> O                      |   | ✓ 13 | 12 | → 13 | 12 | → 13 |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |
| TFI 0.1 $\mu$ l                                      |   | ✓ 1  | 1  | →    |    |      |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Vent 0.002 $\mu$ l                                   |   | ✓    |    |      |    | 1    |   | 1 |   | 1 |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 0.01 $\mu$ l   |   | ✓    |    |      | 1  |      |   | 1 |   | 1 |    |    | 1  |    |    |    |    |    |    |    |    |    |    |
| 0.05 $\mu$ l   |   | ✓    |    |      | 1  |      |   | 1 |   | 1 |    |    | 1  |    |    |    |    |    |    |    |    |    |    |
| rTog 0.5 $\mu$ l                                     |   |      |    |      |    |      |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 5 $\mu$ l  |   |      |    |      |    |      |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Deep Vent 0.002 $\mu$ l                              |   |      |    |      |    |      |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 0.01 $\mu$ l   |   |      |    |      |    |      |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 0.05 $\mu$ l   |   |      |    |      |    |      |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Pfu 0.002 $\mu$ l                                    |   |      |    |      |    |      |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 0.01 $\mu$ l   |   |      |    |      |    |      |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 0.05 $\mu$ l   |   |      |    |      |    |      |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Tne 0.022 $\mu$ l                                    |   |      |    |      |    |      |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 0.01 $\mu$ l   |   |      |    |      |    |      |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 0.05 $\mu$ l   |   |      |    |      |    |      |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |

VF=20%

Preheat all reaction to 70°C, start by addition of  
3'P33 mis - m pl9, add 10  $\mu$ l cycles eq stop at 2 minutes

rTog, Tne TFI use Tog dil buffer  
Pfu, Vent, Deep Vent use NEB Vent dil buffer

To Page N

|  |                 |                  |                 |
|--|-----------------|------------------|-----------------|
| Witnessed & Understood by me,<br>Deena Polanco | Date<br>3/16/95 | Inv nt d by<br>R | Date<br>2-27-95 |
|  |                 | Rec rd d by      |                 |

|  |                 |                                |                 |
|--|-----------------|--------------------------------|-----------------|
| d & Understood by m ,<br><i>Maureen Polansky</i> | Date<br>3/16/95 | Invented by <i>[Signature]</i> | Date<br>2-27-95 |
|  |                 | Rec rd d by                    |                 |

146

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

 $\Delta$  KAc. Effect on pol and exo, The v

From Page No. \_\_\_\_\_

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

5x Mergs (no KAc  
no DMSO  
no Glycerol)  
(at 5x = 100 mM Tricine pH 7.7,  
5 mM Mg(OAc)<sub>2</sub>)

✓ 4

KOAc 0.2 M

✓

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

\*  
0.33 correct. mPA (same as  
p138 0.06 pmol circle/1)

✓ 2

\*  
32P 33 correct 5 μM primer  
(as was done for "Ac" on 13.6)  
10 mM 4 dNTPs  
H<sub>2</sub>O

✓ 0.4

✓ 11.6 10.4 9.6 8.8 7.4 6.6 5.6 4.6 3.8 2.6 1.6 11.6 10.4 9.6 8.8 7.4 6.6 5.6 4.6 3.8 2.6 1.6

vTag 0.001 μ/λ

2

Tne 0.004 μ/λ

2

Tne 2.5 μ/λ

v<sub>f</sub> = 20λ

70°C, 5'

\* 33 correct has  
same 5' end as 23mer sequencing primer

To Page N

Witnessed &amp; Understood by me,

Deborah W. Blazynski

Date

3/16/95

Invented by

Recorded by

Date

3-1-95

ag No. 27 28 29 30 31 32 33

→ ✓

2 3 4 5 6 7 8 9 10 ✓

→ ✓

12.2 11.2 10.2 9.2 8.2 7.2 6.2 5.2 4.2

JX Chung on P 79:

20 mM Tris HCl pH 8.7

1.2 mM MgOAc

80% glycerol

20% DMSO

plus K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> which is varied

$C_f \approx 200 \text{ mM}$  from 0-100 mM in this experiment.

→

→

70°C, 60'

start 11.8

To Page No. \_\_\_\_\_

sed & Understood by me,

Ernest Polansky

Date

3/16/95

Invented by

*[Signature]*

Record d by

Dat

3-1-95

Project No. \_\_\_\_\_

10x PCR same as P140

Book No. \_\_\_\_\_

TITLE is the inhibitory at 7 units ?

148

From Page No. \_\_\_\_\_

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

10x Tag PCR buffer

✓ 2

<sup>32</sup>P 33-mer. mp19 (P146)  
(see P138)

2

50 mM MgCl<sub>2</sub>

✓ 0.6

10 mM JNTPA

✓ 0.4

H<sub>2</sub>O

✓ 14

→ 13

→ 14

r Tag:

0.25 u/l

✓

1

0.5 u/l

✓

1

1

✓

1

1

→ ✓

5

✓

4

5

✓

.6

5

✓

.8

5

✓

1

The

0.25 u/l

- 1

1

0.5

1

1

1

1

1

5

4

4

5

.6

.6

5

.8

5

1

Tag storage buffer

✓ 20 μl

.6 .4 .2

✓ .6 .4 .2

1

✓ .6 .4

preheat to 70°C, add 2 μl <sup>32</sup>P 33-mer mp19 for 30 sec  
kill with 10 μl cyclo sig stops solution  
with 10 mM extra EDTA → ∞ Cf = 20 mM EDTA in stop

To Page No

Witnessed & Understood by me,

Diana Polarp

Date

3/16/95

Invented by

Record d by

Dat

3-3-55

Fig. N. .

**To Page No.\_\_\_\_\_**

**d & Understood by me,**

Date \_\_\_\_\_

$$3 \overline{) 16} 95 -$$

**Invented by**

**Date**

Record d by

read a Polanco

150

Project No. \_\_\_\_\_  
Book No. \_\_\_\_\_

TITLE

AatII #1

<sup>32</sup>P Kinase

From Page No. \_\_\_\_\_

see p 136 for ↑ [primer]

10  $\mu$ M AatII #1  
5x Kinase  
3-<sup>2</sup>P ATP 10 mM  
PNK 1  $\mu$ /1

5 ✓  
4 ✓  
1.0 ✓  
1

(33 pmol ATP)

at 1x  
5 mM MgCl<sub>2</sub> 55 mM  
50 mM KCl

2  $\mu$ l

( $\Rightarrow$  now with 2.5  $\mu$ M primer)

37°C, 30'  $\Rightarrow$  80°C, 5'

↓

mix back into cold primer  
at 5 cold to 1 hot primer

<sup>32</sup>P AatII #1 2.5  $\mu$ M

13.3 20

(2.5  $\mu$ M)

cold AatII #1

16.6 25

(10  $\mu$ M)

30 45  $\mu$ l

(6.67  $\mu$ M)

(MgCl<sub>2</sub> = 2.2)

use 1.5  $\mu$ l / 50  $\mu$ l PCR for 200 nM  
(adds 0.067 mM Mg<sub>2</sub> per PCR (f))

Aagob R. used in 14 PCR's

remove 10  $\mu$ l from each PCR to 5  $\mu$ l stop (up  
and store at -20°C over weekend.

Result: Aagob R.  
did PCR's with Inc

note smear (see EtBr stain  
(P151 photo) is not hot.  
2<sup>o</sup> primer ("AatII #1") is not  
needed for smear

To Page

Witness d & Understood by me,

Doracina Polak

Date

3/16/95

Inv nted by

Rec rd d by

Date

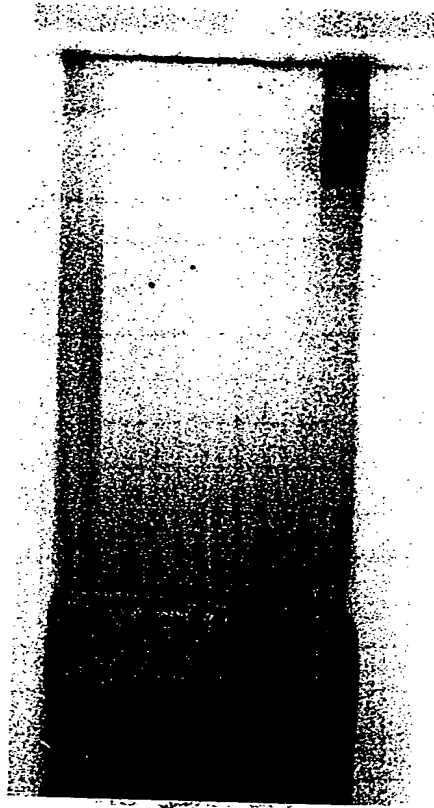
3-3-95

ig No. \_\_\_\_\_

100  
1 2 3 4 5 6 7 8 9 10 11 12  
1 1 2 3 3 4 4 5 6 6 7 7

0.5 x TBE

(lost 2nd, 5th)



To Page No. \_\_\_\_\_

d & Understood by me,

Date

3/16/95

Invented by

R cord d by

Date

2-7-95

Richard Pokany

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

Unit assay for stability of vTox in  
PCR mix. Repeat of assay on P 121

152

From Page No. \_\_\_\_\_

This assay is 33 days after the first assay of 2-3-95.

carry out all assays with exact same procedure  
of P 120 - 122, same MgCl<sub>2</sub> TAPS, KCl mix of P12  
same stock of 'activated' DNA, same 5' u/pl vTox stock  
on P 121 → (of 1-31-95)

(3' p d SP is a new stock of 10 mc/ml on 3-10-95)

To Page N

Witnessed & Understood by m ,

Deena Bolay

Date

3/16/95

Inv nted by

Record d by

Date

3-9-95  
3-8-95

related to Tag  $\Rightarrow$   $\frac{u}{1}$   
 and

Project No. \_\_\_\_\_

B k No. 02

| activity |              |             |       |      | activity |
|----------|--------------|-------------|-------|------|----------|
| idg #1   | 1 23165.00   | } 24896     | .031  | .037 | 84       |
|          | 2 26508.00   |             |       |      |          |
|          | 3 25014.00   |             |       |      |          |
| 2        | 4 24738.00   | } 24616     | .031  | .033 | 94       |
|          | 5 23608.00   |             |       |      |          |
|          | 6 25502.00   |             |       |      |          |
| 3        | 7 23947.00   | } 23577     | .030  | .032 | 94       |
|          | 8 24449.00   |             |       |      |          |
|          | 9 22336.00   |             |       |      |          |
| 4        | 10 19450.00  | } 19801     | .025  | .029 | 86       |
|          | 11 20001.00  |             |       |      |          |
|          | 12 19953.00  |             |       |      |          |
| 5        | 13 21103.00  | } 22158     | .028  | .033 | 85       |
|          | 14 20211.00  |             |       |      |          |
|          | 15 25159.00  |             |       |      |          |
| 6        | 16 19309.00  | } 18853     | .024  | .027 | 89       |
|          | 17 18318.00  |             |       |      |          |
|          | 18 18933.00  |             |       |      |          |
| 7        | 19 22404.00  | } 25532     | .029  | .033 | 87       |
|          | 20 25483.00  |             |       |      |          |
|          | 21 22108.00  |             |       |      |          |
| 8        | 22 20542.00  | } 25507     | .029  | .035 | 85       |
|          | 23 27602.00  |             |       |      |          |
|          | 24 21776.00  |             |       |      |          |
| 9        | 25 22624.00  | } 22051     | .028  | .031 | 90       |
|          | 26 23813.00  |             |       |      |          |
|          | 27 20017.00  |             |       |      |          |
| 10       | 28 10829.00  | } 11703     | .015  | .021 | (70)     |
|          | 29 12483.00  |             |       |      |          |
|          | 30 11798.00  |             |       |      |          |
| 11       | 31 23967.00  | } 24527     | .031  | .032 | 97       |
|          | 32 25056.00  |             |       |      |          |
|          | 33 24557.00  |             |       |      |          |
| 12       | 34 26587.00  | } 25000     | .032  | .034 | 93       |
|          | 35 23432.00  |             |       |      |          |
|          | 36 24980.00  |             |       |      |          |
| 13       | 37 25401.00  | } 24694     | .031  | .031 | 100      |
|          | 38 24104.00  |             |       |      |          |
|          | 39 24576.00  |             |       |      |          |
| 14       | 40 25123.00  | } 25962     | .033  | .035 | 94       |
|          | 41 25545.00  |             |       |      |          |
|          | 42 27217.00  |             |       |      |          |
| 15       | 43 24143.00  | } 25703     | .030  | .032 | 93       |
|          | 44 23491.00  |             |       |      |          |
|          | 45 23474.00  |             |       |      |          |
| Tag      | 46 30440.00  | } 31731     | (.04) |      |          |
|          | 47 31721.00  |             |       |      |          |
|          | 48 30572.00  |             |       |      |          |
| 10       | 49 32938.00  | } 17377     | .022  |      |          |
|          | 50 32985.00  |             |       |      |          |
|          | 51 17357.00  |             |       |      |          |
| det      | 52 17994.00  | } 144943.00 |       |      |          |
|          | 53 16781.00  |             |       |      |          |
|          | 54 144943.00 |             |       |      |          |
| 21       | 55 145358.00 |             |       |      |          |

note #10 is not sufficient. 3. added det be  
 at .01 % Tween 20/NP4 each in Reaction mix

sed & Und rstood by me,

Date

Inv nted by

Date

R corded by

To Page No. \_\_\_\_\_

*enclosed*

3/16/90

*[Signature]*

3-9-85

Test of rule to use 1/600 Tag dil  
between 20-40 min after mixing

Pr j ct N \_\_\_\_\_  
B ok N \_\_\_\_\_

Exhibit 53  
Appl. No. 09/558,421

155

Standard Tag units array as per 120-120

10 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

e# 123 4 5 6 7 8 9  
tube# 123 4 5 6

21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40

10 11 12 13  
7 8 9 10 11 12 13

41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 70 2hr 3hr

# 14 15 16 17 18  
# 14 15 16 17 18

make a standard 1/600 dil

1797  $\mu$ l Tag dil buffer  
3  $\mu$ l 5 u/l tag  
Vortex 5"

use immediately in triplicate for reactions 1, 2, 3 at  
0, 20 sec and 60 sec  
also # 1797 20<sup>22</sup> sit on ice + 2 3 hr before 10)  
EDTA (it is time at 74°C to see if any activity at 0°C.

To Page No. \_\_\_\_\_

ed & Understood by me,

*Michael Polansky*

Date

3/16/95

Invented by

*[Signature]*  
Recorded by

Date

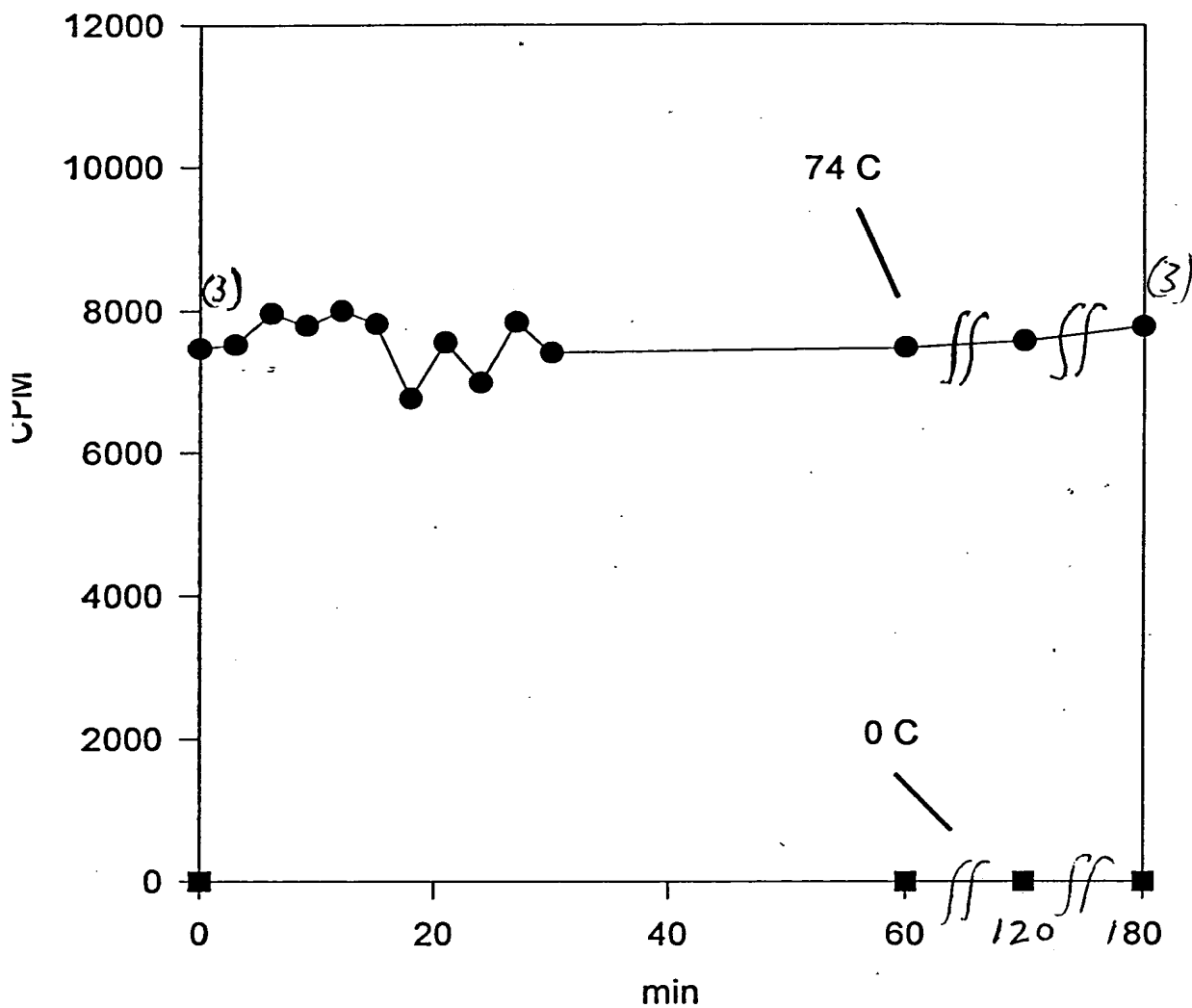
3-15-95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

## Time allowed before assay of Taq dilution



| SAM | CPM |
|-----|-----|
| 1   | 738 |
| 2   | 845 |
| 3   | 705 |
| 4   | 770 |
| 5   | 809 |
| 6   | 802 |
| 7   | 820 |
| 8   | 796 |
| 9   | 692 |
| 10  | 764 |
| 11  | 733 |
| 12  | 801 |
| 13  | 760 |
| 14  | 970 |
| 15  | 765 |
| 16  | 784 |
| 17  | 750 |
| 18  | 828 |
| 19  | 827 |
| 20  | 27  |
| 21  | 66  |
| 22  | 40  |
| 23  | 59  |

less d &amp; Understood by me,

Deena Boland

Date

4/4/95

Invent d by

Record d by

Date

3-15-95

10 Page No.

Tet stock / streak T<sup>+</sup> clones

Project N \_\_\_\_\_

Exhibit 54

Book N \_\_\_\_\_

Appl. No. 09/558,421

157

0.4g

Tetracycline

Sigma crystalline

(not a salt)

40 ml

ETOH

Amp/Tet plates

have BBL Amp plates (100 µg/ml) Vol ~ 15 ml agar

To make 50 µg/ml Tet spread

15 µl 10 mg/ml Tet on each - let sink in > 30 min

25 50 µg/ml Tet in 15 ml agar on plate

streak out cell (glycine) streaks of A.R.

sup 94-95 for stock names

grow at 30°C O/N

start 2 ml O/N from single colonies 3-21-95  
of each in will grow, 100 µg/ml Amp, 50 µg/ml Tet

3-22-95

inoculate 0.4 ml of each O/N into 3.5 ml will grow  
+ 100 µg/ml Amp, 50 µg/ml Tet

shake at 30°C starting at 8:30

To Page No. \_\_\_\_\_

Read & Understood by m ,

Date

Invent d by

Date

Andrea Polansky

4/4/95

Recorded by

3-20-95  
3-21-95

Tet stock / streak T<sup>+</sup> clones

Project No. \_\_\_\_\_

Block No. \_\_\_\_\_

Exhibit 55

Appl. No. 09/558,421

157

0.4g

Tetraacycline

Sigma crystalline

(not salt)

40 ml

ETOH

Amp / Tet plates

have BBL Amp plates (100 µg/ml) Vol. 15 ml agar

to make 50 µg/ml Tet spread

Typ. 10 mg/ml Tet on water - let sink in  $\geq 30$  min

25 50 µg/ml Tet in 15 ml agar on plate

streak out cell (glycerol) stocks of A.R.

sup 94-95 for stock names

grow at 30°C O/N

start 2 ml O/N from single colonies 3-21-95  
of each in will grow, 100 µg/ml Amp, 50 µg/ml Tet

3-22-95

inoculate 0.4 ml of each O/N into 35 ml will grow  
+ 100 µg/ml Amp, 50 µg/ml Tet

shake at 30°C starting at 8:30

To Page No. \_\_\_\_\_

Read & Understood by m ,

Barbara Polansky

Date

4/4/95

Invented by

Recorded by

Date

3-20-95  
3-21-95

Tet stock / streak T+1 clones

Page N

0.4g Tetraacycline Sigma crystalline (not salt)  
40 ml ETOH

Amp/Tet plates

have BBL Amp plates (100 µg/ml) Vol ~ 15 ml agar  
To make 50 µg/ml Tet spread

15 µl 10 mg/ml Tet on each - let sink in ≥ 30 min  
20  
50 µg/ml Tet in 45 ml agar on plate

streak out cell (glycerol) stocks of A.R.

SUP 94-95 for stock names

grow at 30°C O/N

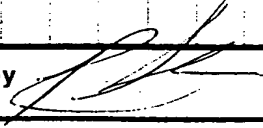
start 2 ml O/N from single colonies 3-21-95  
of each in will grow, 100 µg/ml Amp, 50 µg/ml Tet

3-22-95

inoculate 0.4 ml of each O/N into 35 ml will grow  
+ 100 µg/ml Amp, 50 µg/ml Tet

shake at 30°C starting at 8:30

T Page N

|   |                |   |                            |
|---|----------------|---|----------------------------|
| ed & Understood by m ,<br>Sandra Polansky | Date<br>4/4/95 | Invent d by<br> | Date<br>3-20-95<br>3-21-95 |
| R cord d by                               |                |   |                            |

From Page No. \_\_\_\_\_

3-22

30°C  
Start 8:30  
12:30 .274  
2:00 .770

ASSD

↓ 42°C, 15 min

↓ 1 hr 37°C

3-23

extract w/ 55°C heat for FrI is  
same as p 95 and P115, 6

pol assay is same as PST except add just  
2 µl FrI' / 97 µl Rxn cocktail  
and remove time points  
array 1 2 5 µl of ~~Rxn~~ FrI' in 50 µl  
Taq unit array (using TFI buffer system  
for 5 min at 74°C

|       | <u>3-23-95</u> | <u>12-15-94</u> |
|-------|----------------|-----------------|
|       | %              |                 |
| 106   | (100)          | 64              |
| 107 H | 87             | 92              |
| 108 H | 86             | (100)           |
| 152   | 83             | 59              |
| 151   | 56             | 95              |
| 202   | 20             | 26              |
| 109   | 2              | 11              |

To Page No. \_\_\_\_\_

Witnessed &amp; Understood by me,

Deena a Polkamp

Date

4/4/95

Invented by

Rec rd d by

Date

3-23-95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

158

From Page No. \_\_\_\_\_

3-22.

30°C  
Start 8:30  
12:30 .274  
2:00 .770

ASD  
↓ 42°C, 15 min

↓ 1 hr 37°C

3-23

extract and 55°C heat for FrI is  
same as p 95 and p 115, 6

pol assay is same as PST except add just  
2 µl FrI' / 97 µl Rxn cocktail  
and remove time points  
array 1 2 5 µl of FrI' in 50 µl  
Toy unit assay (using TFI buffer system)  
for 5 min at 74°C

|      | 3-23-95 | 12-15-94 |
|------|---------|----------|
| 106  | (100)   | 64       |
| 107H | 87      | 92       |
| 108H | 86      | (100)    |
| 152  | 83      | 59       |
| 151  | 56      | 95       |
| 202  | 20      | 26       |
| 109  | 2       | 11       |

To Page No

Witnessed & Understood by me,

Deena a Pokany

Date

4/4/95

Invented by

Record d by

Dat

3-23-95



Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

5% PEI stock

160

From Page No. \_\_\_\_\_

Same as P155, 6 except ~~use~~ instead  
of using complete Tag ext buffer (P167, 3)  
just use 50 mM Tris HCl pH 7.5, 1 mM EDTA

A = 50 mM Tris pH 7.5 275 ml  
1 mM EDTA

(55264A BRG) PEI 50% 50 ml

stir  $\geq 30'$

adjust pH to 7.4 with HCl  
add A to  $V_f = 500$  ml

To Page 1

Witnessed & Understood by me,

Date

Invented by

Dat

Diana Bolamp

4/4/95

R corded by

3-24-95

grow 2L TF1-106

Exhibit 59  
Project No. \_\_\_\_\_  
Book No. \_\_\_\_\_  
Appl. No. 09/558,421

161

ag N

make 2x LB (il 40 g/L of LB broth base  
eg as per P 119, b for D. + E

make 20 ml O/N of TF1-106  
in LB + 100 µg/ml Amp, 30 µg/ml Tet  
(Morgan uses 15-20 µg/ml Tet)

10 mg/ml

Ampicillin (Sigma A-9518)

2 g

H<sub>2</sub>O

200 ml

filter sterilize

inoculate 10 ml O/N / 1L LB

start shaking at 30°C at P: 20 AM  
start 7:20 AM  
12:30 PM  
OD<sub>550</sub> 0.567

induce each at 42°C, 15' - by rapidly  
bringing up to 42°C in hot tap water bath  
and then 42°C in water shaker 15'

37°C 1 hr in air shaker  
cool in ice water bath

and 1 hr 37°C at 2:05 and 2:35 respectively

OD<sub>550</sub> final = 0.812 ⇒ recovered 5.64g cells  
(spin 51L GSS 45 min)

To Page No. \_\_\_\_\_

Read & Understood by me,

Date

Invented by

Date

Deena Polansky

4/4/95

Recorded by

3-26-95

3-27-95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

5 buffers for 50g TFI prep

162

Form Page No. \_\_\_\_\_

follow v Tag PRP 91342. PRP \* except for a 2m KCl in buffer B.

2L  
buffer B

1L  
buffer C

1M Kphos monobasic ✓ x 34.2

17.1 ml

1M Kphos dibasic ✓ x 15.7

7.9 ml

glycerol ✓ x 160

80

KCl ✓ x 7.46g  
(50mm)

149.12  
(2m)

EDTA 0.5M x x 0.4ml

0.2 ml

BME 14.3M ✓ x 700  $\mu$ l

350  $\mu$ l

H<sub>2</sub>O 2L

1L

buffer C is 2mK  
here in order to do  
elution point - m  
in Tag PRP C.  
700 mm KCl

To Page N

Witnessed & Understood by me,

Deanna Polanco

Date

4/4/95

Inv nted by

Rec rd d by

Date

3-27-95

AmSO<sub>4</sub> optimization for TFI  
(can see P 22, 7 for Tag)

Project No. \_\_\_\_\_

Exhibit 61

Appl. No. 09/558,421

B ok No. \_\_\_\_\_

163

ag N —

3.64 g

TFI cells (P161)

18 ml

Tag ext buffer (P167,3)

sonicate

heat treat

75°C, 30 min

PEI

adjust to 200 mM NaCl

Vol = 20 ml so add 1.33 ml NaCl 3M

add 5% PEI (P160) to C<sub>f</sub> = 0.4%  
stir 15 min (1.7 ml 5% PEI)

Centrifuge

SS 34 15' 15 K

residual 17 ml

supr

= Fr I' / PEI

start 11:30 AM

stir AmSO<sub>4</sub> in 15', spin  
SS 34 15 K, 15 min

2 AmSO<sub>4</sub>

of salt

1 Fr I' / PEI

2.45 g

25

2

.493 g

30

3

.51

35

4

.51

40

5

.527

45

6

.527

50

7

.544

55

8

.561

60

9

.561

65

10

.578

70

To Page No. \_\_\_\_\_

Read & Understood by me,

Date

Inv nted by

Dat

rebecca Polansky

4/4/95

Recorded by

3-29-95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

Pol assay of AmSO<sub>4</sub> supe

64

from Page No. \_\_\_\_\_

assay 2  $\mu$ l of  $1/100$  dil of each supe in 48.  
ml R<sub>xn</sub> mix (P-120) for 15 min at 74°  
kill with 10  $\times$  EDTA  
spot 40 $\lambda$

| Bradford              |       | mg/ml       | To Protein remaining |
|-----------------------|-------|-------------|----------------------|
| I / PEI               | 20    |             |                      |
| AmSO <sub>4</sub> 25% |       | .412 (0.39) | 100                  |
|                       |       | .450 0.40   | 102                  |
| 30                    |       | .430 .37    | 97                   |
| 35                    |       | .449 .40    | 102                  |
| 40                    |       | .425 .37    | 97                   |
| 45                    |       | .405 .36    | 92                   |
| 50                    |       | .370 .33    | 85                   |
| 55                    |       | .347 .31    | 79                   |
| 60                    |       | .340 .30    | 77                   |
| 65                    |       | .277 .25    | 64                   |
| 70                    |       | .242 .21    | 54                   |
| I-PEI/70%             | 20/20 | .604        |                      |
| BSA 1mg/ml            | 1     | .105        |                      |
|                       | 2     | .176        |                      |
|                       | 4     | .263        |                      |
|                       | 6     | .382        |                      |
|                       | 8     | .474        |                      |
|                       | 10    | .546        |                      |

| AmSO <sub>4</sub> % | CPM1       | $\mu$ /ml = 0.64 |   |
|---------------------|------------|------------------|---|
| 0 1                 | (11483.00) | (100)%           | $\Rightarrow$ 10,980 $\mu$ /17ml FRI $\Rightarrow$ 3017 $\frac{\mu}{g}$ cells |
| 25 2                | 10706.00   | 93               | (3.64 $\frac{\mu}{g}$ cells used)   |
| 30 3                | 11635.00   | 100              |   |
| 35 4                | 10329.00   | 90               |   |
| 40 5                | 7609.00    | 66               |   |
| 45 6                | 803.00     | 7                |   |
| 50 7                | 465.00     | 4                |   |
| 55 8                | 514.00     | 4                |   |
| 60 9                | 258.00     | 2                |   |
| 65 10               | 313.00     | 3                |   |
| 70 11               | 230.00     | 2                |   |
| Blank 12            | 126.00     |                  |   |
| 21 13               | 106668.00  |                  |   |

conclude 45% AmSO<sub>4</sub>  
brings down > 90% in

$\Rightarrow$  66.7 cpm

BSA

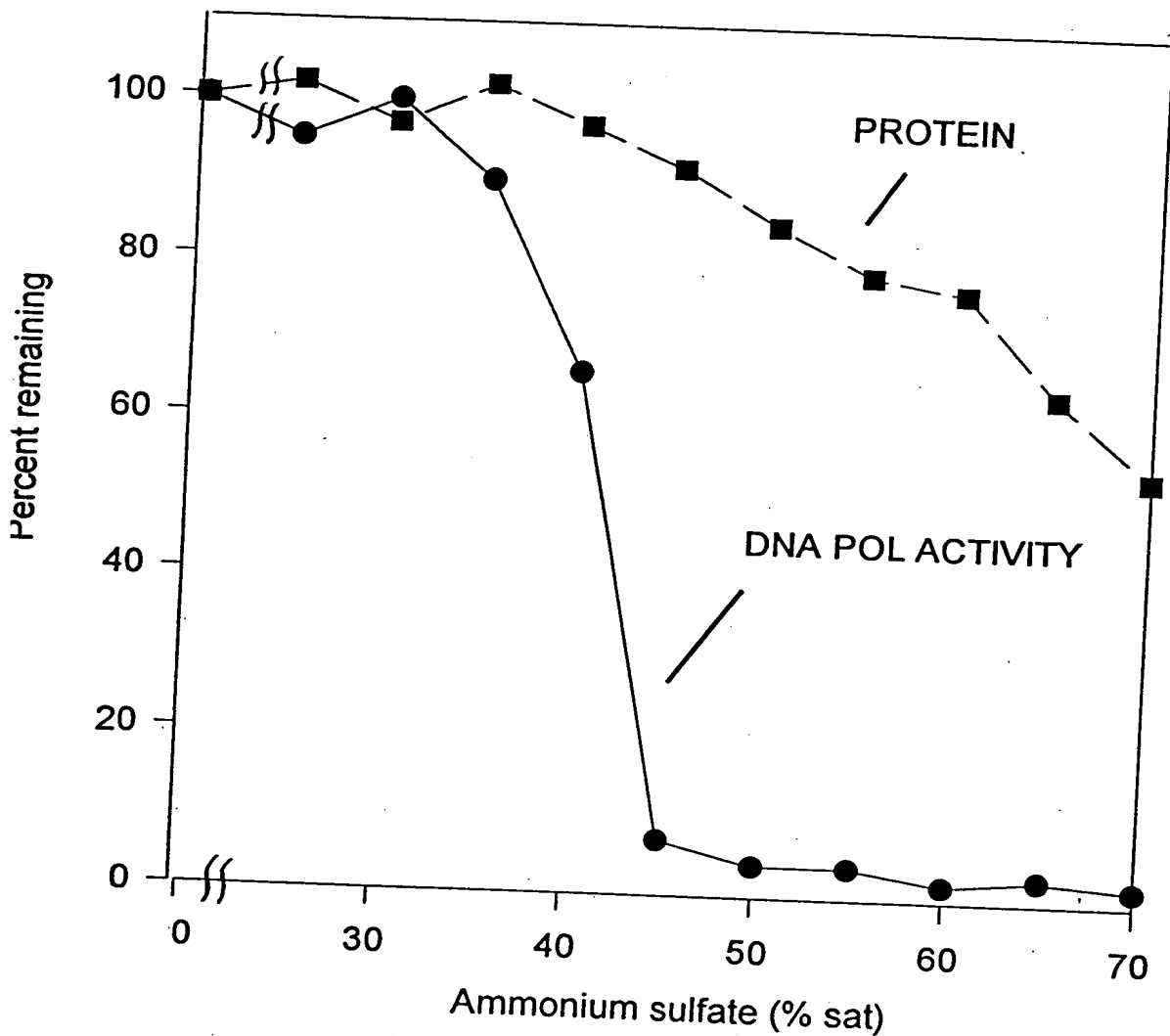
Project N

Bo k N

165

ig No

## Precipitation of Tfl DNA polymerase



To Page No.

sed &amp; Und rstood by me,

Dat

Inv nt d by

Date

Sue A. Polansky

4/4/95

Recorded by

166

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE prep a 180 ml sepharose 200

From Page No. \_\_\_\_\_

in a Pharmacia 2.6 XK

1. make slurry of cold sepharose 200 - want a  
1.5 x vol of pack vol

2.  $1.5 \times 200 \text{ ml slurry} = 300 \text{ ml}$

3. add 100 ml col buffer (buffer B p 162)  
so vol now = 2 x pack vol

use reservoir  
and gravity flow (got 50 ml/min with effluent tube 2  
below bottom of column and 100.  
in reservoir) -  $\sim 1/4 \text{ vol vol/hr}$

bed volume ended up  $\sim 185 \text{ ml}$  (2.6 cm x 35 cm)

5 well 25 ml (bed vol) of Blue sepharose (Ph  
L65, in buffer B (p 162))

since dry swells 4 x 6.25 g

To Page No. \_\_\_\_\_

Witnessed &amp; Understood by me,

Deborah Polansky

Date

4/4/95

Invented by

Record d by

Date

4/31/95

Stability unit assay for Tag  
series as p 121 and 152

Project No. \_\_\_\_\_  
Book No. \_\_\_\_\_

Exhibit 64  
Appl. No. 09/558,421

167

No. \_\_\_\_\_

note stability study tube # 10 (unit assay # 27-30)  
get 0.01% Taren 20/NP40 each added to reaction  
by adding 0.5 ml of 1% stock

tube 51-56 =

Dextran

1.25 mg/ml

1 ml

Cf

0.025

2.5

1

0.05

5

1

0.1

10

1

0.2 mg/ml

10

2

0.4

10

3

0.8

51 19252.00  
52 18303.00  
53 18777.00  
54 18582.00  
55 17015.00  
56 17487.00  
57 267.00  
58 104554.00

⇒ 65.3 cpm/pmol

To Page No. \_\_\_\_\_

ed & Understood by me,

reera Pokay

Date

4/13/95

Invented by

Recorded by

Date

4-4-95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

u/λ on P122

From Page No. \_\_\_\_\_

| SAM | CPM1     | u/λ assuming<br>rtay in .044/λ in 1/12581 | o to<br>o/u/λ P122 |
|-----|----------|---|--------------------|
| 1   | 13329.00 |   |                    |
| 2   | 14243.00 | .032                                      | .037               |
| 3   | 14542.00 |   | 8604               |
| 4   | 14132.00 |   |                    |
| 5   | 13839.00 | .032                                      | .033               |
| 6   | 13367.00 |   | 97                 |
| 7   | 14361.00 |   |                    |
| 8   | 14576.00 | .033                                      | .032               |
| 9   | 14684.00 |   | 97                 |
| 10  | 11765.00 |   |                    |
| 11  | 12054.00 | .027                                      | .029               |
| 12  | 11446.00 |   | 93                 |
| 13  | 13666.00 |   |                    |
| 14  | 13091.00 | .030                                      | .033               |
| 15  | 12913.00 |   | 91                 |
| 16  | 10381.00 |   |                    |
| 17  | 10049.00 | .024                                      | .027               |
| 18  | 10787.00 |   | 89                 |
| 19  | 16428.00 |   |                    |
| 20  | 14956.00 | .034                                      | .033               |
| 21  | 15556.00 |   | 103                |
| 22  | 15357.00 |   |                    |
| 23  | 14468.00 | .033                                      | .035               |
| 24  | 13489.00 |   | 94                 |
| 25  | 14348.00 |   |                    |
| 26  | 12027.00 | .030                                      | .031               |
| 27  | 13354.00 |   | 97                 |
| 28  | 9416.00  |   |                    |
| 29  | 8913.00  | .021                                      | .022               |
| 30  | 9177.00  |   | 100                |
| 31  | 13920.00 | .032                                      | .032               |
| 32  | 13672.00 |   | 100                |
| 33  | 13373.00 |   |                    |
| 34  | 14628.00 |   |                    |
| 35  | 13728.00 | .033                                      | .034               |
| 36  | 15178.00 |   | 97                 |
| 37  | 14616.00 |   |                    |
| 38  | 14209.00 | .034                                      | .031               |
| 39  | 15366.00 |   | 109                |
| 40  | 14402.00 |   |                    |
| 41  | 14584.00 | .034                                      | .035               |
| 42  | 15003.00 |   | 97                 |
| 43  | 12819.00 |   |                    |
| 44  | 13391.00 | .030                                      | .032               |
| 45  | 13180.00 |   | 94                 |
| 46  | 16169.00 |   |                    |
| 47  | 18733.00 |   |                    |
| 48  | 18552.00 |   |                    |
| 49  | 16396.00 |   |                    |
| 50  | 12907.00 |   |                    |

17463 ave (.044/λ)  
by definition

\*for #10, use .022 u/λ on P153 for  
dot added as 0 string point

To Page No.

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Invented by

Date

Rec rd d by

Deanna Polansky

4/13/95

4-4-95

| je No. _____ | P12 in<br>other point | 1 month | 2 months | 4 months |
|--------------|-----------------------|---------|----------|----------|
| .1% TN       |                       | 84      | 86%      | 87       |
| .2% BJ       |                       | 94      | 97       | 98       |
| .2% TX       |                       | 94      | 97       | 106      |
| .01% TN      |                       | 86      | 93       | 93       |
| .02% BJ      |                       | 85      | 91       | 105      |
| .02% TX      |                       | 89      | 89       | 88       |
| 1% TN        |                       | 88      | 103      | 104      |
| 2% BJ        |                       | 83      | 94       | 91       |
| 2% TX        |                       | 90      | 97       | 99       |
| No detergent |                       | 95      | 95       | 91       |
| 1.1X         |                       | 97      | 100      | 94       |
| 5X           |                       | 93      | 97       | 100      |
| 2x R2GE 0.1% |                       | 100     | 109      | (35)     |
| 2x Tf 1.01%  |                       | 94      | 97       | 89       |
| 2x Vent      |                       | 93      | 94       | 97       |

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aaa Polay

Date

4/13/95

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Recorded by

Dat

4-4-95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

Tf1 growth of 4-4-95

70

From Page No. \_\_\_\_\_

got ~ 0.6-0.8 g cells from 50ml samples taken at 0 1 2 3 4 hr post induction for 10L pen of minimal media (001R) and the same for buffered rich (002R) plus 50ml at end (~4hr post induction) for (002R) plus 114g bulk  
[chipped off 0.55g of bulk for 4hr 002R sample]

Resuspend cells in Tox ext buffer (P167, 3)  
add 25 ml ext buffer  $\Rightarrow$  0.9 g cells/ml

sonicate 3 x 10 sec max setting microtip

microport 15 min, sup = Fr I

90°C 5 min

microport 15 min = sup = Fr I'

22.2  $\frac{\text{cpm}}{\text{pmol}}$   
P167

pol assay is 2  $\mu\text{l}$  of  $\frac{1}{100}$  and  $\frac{1}{500}$  dil of Fr I'

| hr after induction | cpm | pmol    | $\mu\text{l}$ | graffed<br>Assay vol | mg/ml | $\mu\text{g}$ |
|--------------------|-----|---------|---------------|----------------------|-------|---------------|
| 0.50               | 1   | 874.00  | -21           | 0.31                 | 0.548 | 1.03 306      |
| 1                  | 2   | 440.00  |               |                      |       |               |
|                    | 3   | 6172.00 |               |                      |       |               |
|                    | 4   | 1538.00 | 2.6           | .677                 | 1.28  | 2029          |
| 2                  | 5   | 5174.00 |               |                      |       |               |
|                    | 6   | 1058.00 | 1.8           | .648                 | 1.23  | 1467          |
| 3                  | 7   | 6330.00 |               |                      |       |               |
|                    | 8   | 1537.00 | 2.6           | .670                 | 1.27  | 2050          |
| 4                  | 9   | 5734.00 |               |                      |       |               |
|                    | 10  | 1206.00 | 2.1           | .662                 | 1.25  | 1675          |
| C                  | 11  | 1058.00 | 0.58          | .639                 | 1.21  | 314           |
|                    | 12  | 324.00  |               |                      |       |               |
| 1                  | 13  | 3961.00 |               |                      |       |               |
|                    | 14  | 1227.00 | 2.1           | .672                 | 1.29  | 1626          |
| 2                  | 15  | 4250.00 |               |                      |       |               |
|                    | 16  | 1009.00 | 1.7           | .700                 | 1.33  | 1282          |
| 3                  | 17  | 4730.00 |               |                      |       |               |
|                    | 18  | 1046.00 | 1.8           | .641                 | 1.21  | 1483          |
| 4                  | 19  | 3435.00 |               |                      |       |               |
|                    | 20  | 763.00  | 1.3           | .734                 | 1.27  | 1018          |

To Page No. \_\_\_\_\_

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Deena Polay

Date

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4-5-95

114 g *Thomomys* fl. ~~cells~~  
mini g granlin

Project No. \_\_\_\_\_ Exhibit 66  
Book N. \_\_\_\_\_ Appl. No. 09/558,421

171

cells 9504-02-767-03-002R  
(4 hr after induction)

Follow rTag PRP Document # 91342. PRP

114 gram cells

450 ml Tag extract buffer (buffer A)  
with fresh <sup>5 mM</sup> BME + 50  $\mu$ g/ml PMSF

~ 564 ml ( $\approx 0.2$  g cells/ml)

one pass *Thomomys* mini granlin 10,000 PSI

heat to 75°C ~~50~~ (~15')  
in 90°C water bath

15 min more at 75°C  $\rightarrow$  cool in ice slurry

Adjust NaCl to 200 mM

have 550 ml Fr I' (ie after heating)

add 6.43 g NaCl

PEI adjusted to 0.4% by adding

47.8 ml 5% PEI pH 7.4 slowly, then  
stir 15 min more

To Page No. \_\_\_\_\_

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seen a Polars

4/13/95

Recorded by

4-7-95

Spin 30 min in GSA 13,000 RPM

5 Ammonium sulfate

Recovered 506.6 ml of Fr I' / PEI  
want 4.75%  $\text{Am}(\text{SO}_4)_3$  saturation

$$= 295.5 \text{ g} / \text{L}$$

so add 149.7 g to 506.6 ml Fr I' / P

add slowly, stir 30 min more

centrifuge GS-3, 2500 rpm, 60 min

—  $\text{Am}(\text{SO}_4)_3$  pellet was coming off side of bottle  
after 60 min spin  
looks like <sup>density</sup> pellet & <sup>density</sup> solution

will try 2 hr at 13000 RPM in GSA

27000 g compared to ~12000 for GS-3

and smaller bottles (~138 ml / bottle in 4 bottle  
— result: pellets still floating

— collected ppts in filter and mixed into  
32 ml of clear filtrate

— spin 30 min in SS-34 1PK

and spin 1 ml of 32 ml total in microfuge for  
unit assay.

3 N

Try diluting 1:1 the suspended  $\text{AmSO}_4$  ppt  
+ Try ext buffer lacking glycerol (ie 50 mM  
Tris HCl pH 7.5, 10 mM KCl) plus 47.5%  
saturated  $\text{AmSO}_4$ . ie, the only effect is  
to reduce Cf of glycerol from 8% to 4%  
to see if ppt will pellet better

Result: ppt floats in 4% and also w/o glycerol!  
it does ~~not~~ sink in H<sub>2</sub>O

Result:  
see P 176 - cells induced only 1 hr don't  
have problem of  $\text{AmSO}_4$  pellets not sinking  
must be too many lipids in cells used here  
from 4 hr fermentation time point!

To Page No. \_\_\_\_\_

d &amp; Understood by m ,

Date

Invented by

Date

sue a. P. Camp

4/13/85

Recorded by

7-845

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

Stability of Toy at room Temp

74

from Page No. — see P154, 3-13-95. Samples have been at room Temp 24  
assay same as P121, 152.

0.5 M  
T cps 200 ml pH 9.3 (at room Temp)  
(243.3 mW) (Seymour T<sup>cut</sup> - 5130)

24.33g + ~140 ml H<sub>2</sub>O  
2m KOH to pH 9.5

H<sub>2</sub>O to 200 ml

tube # 1 - 30 is stability study 1E-15E in duplicate  
note tubes 19, 20 (no detergent) gets 0.5 ml of 1% Tween 20/R  
24, 24 in the reactions.  
(ie sample is stability study)

Witnessed & Understood by me,  
Dorinda A. Polay

Date  
4/13/95

Invented by

Recorded by

Date

7-11-95

To Page No

Results of P114  
Crack TFI borne as P

Project N \_\_\_\_\_  
Book No. \_\_\_\_\_

175

| SAM       | CPM1      | ave   | $\frac{a}{1}$ | from TIME | $\frac{\sigma}{\sigma_0}$ |
|-----------|-----------|-------|---------------|-----------|---------------------------|
| 1         | 26896.00  | 26508 | .0471         | 1.00      | of P121 values            |
| 2         | 27150.00  |       |               | 1.00      |                           |
| 3         | 26135.00  |       |               | 1.00      |                           |
| 4         | 25462.00  |       |               | 1.00      |                           |
| 5         | 26896.00  |       |               | 1.00      |                           |
| 1 { 6     | 22094.00  | 22048 | .033          | .037      | 89                        |
| 7         | 22002.00  |       |               | 1.00      |                           |
| 2 { 8     | 22874.00  | 22955 | .035          | .033      | 106                       |
| 9         | 23036.00  |       |               | 1.00      |                           |
| 3 { 10    | 21345.00  | 22335 | .034          | .032      | 106                       |
| 11        | 23325.00  |       |               | 1.00      |                           |
| 4 { 12    | 17420.00  | 17637 | .027          | .029      | 93                        |
| 13        | 17853.00  |       |               | 1.00      |                           |
| 5 { 14    | 19189.00  | 19840 | .030          | .031      | 91                        |
| 15        | 20491.00  |       |               | 1.00      |                           |
| 6 { 16    | 14064.00  | 14229 | .021          | .021      | 78                        |
| 17        | 14394.00  |       |               | 1.00      |                           |
| 7 { 18    | 19638.00  | 20655 | .031          | .033      | 94                        |
| 19        | 21673.00  |       |               | 1.00      |                           |
| 8 { 20    | 22693.00  | 20245 | .031          | .031      | 89                        |
| 21        | 17798.00  |       |               | 1.00      |                           |
| 9 { 22    | 17031.00  | 18271 | .028          | .031      | 90                        |
| 23        | 19511.00  |       |               | 1.00      |                           |
| 2N { 24   | 804.00    |       |               | .022      | 0                         |
| 25        | 710.00    |       |               | 1.00      |                           |
| 11 { 26   | 17770.00  | 18729 | .028          | .032      | 88                        |
| 27        | 19687.00  |       |               | 1.00      |                           |
| 12 { 28   | 166725.00 |       |               | .054      |                           |
| 29        | 170523.00 |       |               | 1.00      |                           |
| 13 { 30   | 19772.00  | 19521 | .030          | .031      | 97                        |
| 31        | 20070.00  |       |               | 1.00      |                           |
| 14 { 32   | 21891.00  | 19376 | .029          | .031      | 85                        |
| 33        | 16862.00  |       |               | 1.00      |                           |
| 15 { 34   | 24156.00  | 22789 | .034          | .032      | 106                       |
| 35        | 21422.00  |       |               | 1.00      |                           |
| BK00 { 36 | 1454.00   |       |               | 1.00      |                           |
| 2A { 37   | 134586.00 |       |               | 1.00      |                           |

To Page No. \_\_\_\_\_

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Date

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Date

Recorded by

Michael P. King

5/1/95

[Signature]

4.11.95

176

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

Crack TFI same as P 171

From Page No. \_\_\_\_\_

These cells were grown for post induction

cells are 9504-10-767-03-003 R

grown 4-11-95

resuspended 110 g cells in 440 ml (at room  
 ext buffer (P167, 3) but no detergent  
 10,000 PSI on minigolfer, 1 pass  
 Bring to 75°C in 90°C water (~10 min)  
 75°C for 15 min more.  
 cool in ice slurry

Add NaCl to 200 mM Cf

Fr I vol = ~~510~~ 510 ml  
 so add 5.96 g NaCl

add PEI (5% stock pH 7.4) to Cf = 0.

(used 0.40% last time (P171) but want to get as  
 as much DNA as possible

add 50.4 ml 5% PEI to 510 ml Fr I + N.

⇒ Cf = 0.45%

add PEI dropwise and  
 stir 15 min more

spin GSA 13,000 RPM 30'

recovered 495 ml sup ( = Fr I' / 1

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Date

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Date

To Page N

Domena Polans

5/1/95

Record d by

4-13-95

P116 continued  
Experiment done on P. 123

Project N. \_\_\_\_\_ Exhibit 70  
Book No. \_\_\_\_\_ Appl. No. 09/558,421

117

Page N

Still Needed 3

cut with Ord I to see if full length lac Z is present  
(assuming either AFI III or Aat II recognition region  
had a point mutation generating). Therefore the "410" and "465" bp

miniprep # 54, 58, 64, 73, 87, 98, 103, 108, 113, 125

plus Aat II, AFI III

cut with 55+ I to see if R1 site in MCS was  
a point mutation (or very small deletion  
(see on P107 at bottom) resulting in the "90mers"

miniprep # 3, 29

Recut with 17 µl miniprep and load 30 µl?

25 µl reaction  
to try to resolve the "No results"

miniprep # 20, 39, 71, 74, 75, 76

To Page No. \_\_\_\_\_

Read & Understood by me,

me a Bolanos

Date

2/16/95

Invented by

Recorded by

Date

1-31-95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

Sephacryl

200

From Page No. \_\_\_\_\_

resuspended entire Am. S<sub>2</sub> pellet in buffer B (P.  
added 3 ml to ~ 1 ml pellet.  
tributyl

spin SS34, 13 K RPM, 5 min

odd ~ 20  $\mu$ l buffer B  $\pm$  pellet  
respin  $\rightarrow$  20  $\mu$ l buffer B more

need to microfuge 15 min to clarify

$V_f = 3.5$  ml ( $\approx 1.9\%$  of 180 ml G100 col)

Load on 180 ml sephacryl 200

elute with  $\frac{1}{2}$  col vol/hr buffer B (ie 1.5 ml/min)

note vol started coming off  
column ~ 98 ml

98 ml / 180 ml col vol  $\approx 54\%$  col vol

To Page N. \_\_\_\_\_

Inness d &amp; Und rsto d by me,

Deanna Polans

Date

5/1/95

Invented by

Record d by

Date

4-18-95

**PAGE 179 OF NOTEBOOK WAS BLANK**

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

Standard  
TFI unit assay

From Page No. \_\_\_\_\_

mix used by epicenter same as tag unit assay (P125)  
except only 160  $\mu\text{g}$  DNA/ml instead of 500  $\mu\text{g}$   
in Rxn

0.5 M TAPs pH 9.3  
1 M MgCl<sub>2</sub>  
3 M KCl

A1500  $\mu\text{l}$ 60  $\mu\text{l}$ 500  $\mu\text{l}$ V<sub>A</sub> = 2,060  $\mu\text{l}$ 

"TFI Rxn mix"

A229  $\mu\text{l}$ 

✓

10 mM dNTP

66.7  $\mu\text{l}$ 

✓

3.7 mg/ml DNA

144.2  $\mu\text{l}$ 

✓

10 mM <sup>32</sup>P dCTP6  $\mu\text{l}$ 

✓

H<sub>2</sub>O

2754

3.2 ml

use 48  $\mu\text{l}$  / 50  $\mu\text{l}$  reaction

To Page 1

Witnessed &amp; Understood by me,

Deborah Polansky

Date

5/1/95

Invented by

Recorded by

Date

4-17-95

ag N. Tube #  
 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22  
 Rxn mix p180 4  $\mu$ l  
 PEI  $\frac{1}{100}$  dil 2  $\mu$ l  
 reamphur  $\frac{1}{10000}$  dil 2  $\mu$ l  
 yeast cell fractions  $\frac{1}{1000}$  dil 2  $\mu$ l  
 # 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20  
 1  $\mu$ l  $\frac{1}{100}$  dil 2 -  
 18095  $\mu$ l = 50  $\mu$ l, 74°C, 15 min

2A 1/100  
Am 504 sup  
(2.4 m m  
Am 504 in  
section)

|       | SAM | CPM1     | pmol | u/ml  | total units  |
|-------|-----|----------|------|-------|--|
| PEI   | 1   | 4110.00  | 163  | 2.45  | $1.21 \times 10^6$ units in 495 ml                         |
| yeast | 2   | 6087.00  |      | 362.9 | $1.28 \times 10^6$ in 3.5 ml AmSO <sub>4</sub> resuspended |
| 4     | 3   | 308.00   |      |       | fraction   |
| 5     | 4   | 356.00   |      |       | pool 7-12  |
| 6     | 5   | 678.00   |      |       | = 18 ml total  |
| 7     | 6   | 3373.00  |      |       |  |
| 8     | 7   | 8181.00  |      |       |  |
| 9     | 8   | 11817.00 |      |       |  |
| 10    | 9   | 9111.00  |      |       |  |
| 11    | 10  | 8925.00  |      |       |  |
| 12    | 11  | 5943.00  |      |       |  |
| 13    | 12  | 2583.00  |      |       |  |
| 14    | 13  | 1385.00  |      |       |  |
| 15    | 14  | 773.00   |      |       |  |
| 16    | 15  | 351.00   |      |       |  |
| 17    | 16  | 299.00   |      |       |  |
| 18    | 17  | 304.00   |      |       |  |
| 19    | 18  | 245.00   |      |       |  |
| 20    | 19  | 407.00   |      |       |  |
| 21    | 20  | 2651.00  | 105  | 1.58  | (expected only 1 u/ml stock from epicenter)                |
| 22    | 21  | 358.00   |      |       |  |
| 23    | 22  | 818.00   |      |       |  |
| 24    | 23  | 60259.00 |      |       |  |
| 25    |     |          |      |       |  |

37.7 cpm/pmol

for ~~fr 7-11~~<sup>7-12</sup> = average of  $\sim 8000$  cpm for 1  $\mu$ l  
 $\Rightarrow 47.7$  u/ $\mu$ l  $\Rightarrow$  859,000 total units / 1  $\mu$ l  
 or  $\sim 72\%$  recovery from Fr I' / PEI

182

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

Blue sepharose

From Page No. \_\_\_\_\_

load pooled sepharose 200 fractions #7-12 (18 ml V to  
on 20 ml Blue at 0.35 ml/min (~1 col vol)  
wash 5 col vol O/N at 0.16 ml/min buffer  
gradient is 400 ml vol 50 mM - 1 M KCl  
(use buffer B-C - p162)  
at 3 col vol/hr = 1 ml/min, 6 ml fraction

Buffer

1M Tris pH 7.5  
0.5M EDTA  
Glycerol  
3 ME  
KCl

\* D

200 ml ✓✓  
1.6 ml ✓✓  
640 ml ✓✓  
2.8 ml ✓✓  
29.8g ✓✓

PL

(50 mM KCl)

E

25 ml 12.5 ✓✓  
0.2 ml 0.1 ✓✓  
80 ml 40 ml ✓✓  
350.2 ml 1175 ✓  
142g 74.5g ✓

1E

2M KCl

(note buffer D is  
75 mM KCl in Tris Pop 91342)  
but only 50 mM here)

enter "2" to set to bank 2  
then 5

HOD 5 BANK 2

.00 CONC 1B 0 0  
.00 CONC 2B 0 0  
.00 ML MIN 1  
00 5 1

400 —  
400 —

still 1

a

sed & Underst od by m ,

Date

5/1/95

Invented by

Recorded by

Date

4-19-95

To Page N

ge N — pool Blue fractions 24-32 based on UV profile

$V_f = 54 \text{ ml}$

Dialyze against 5 L buffer D (PIB2) O/N  
 recovered ~ 60 ml

Conductivity

10  $\mu\text{l}$  in 1 ml  $\text{H}_2\text{O}$

in buffer D  
 in cell effluent  
 to equilibration O/N

101  $\mu\text{S}$  = 10.1 mS  
 98  $\mu\text{S}$  = 9.8 mS

Dialysate

99

9.9 mS

(can see P 41 where results are similar)  
 for Toq

To Page No. \_\_\_\_\_

ed & Understood by me,

*maria Polansky*

Date

5/1/95

Invented by

Recorded by

Date

4-20-95

**PAGE 184 OF NOTEBOOK WAS BLANK**

# Heparin AF (20ml vol)

Pr j ct No. \_\_\_\_\_  
Bo k N . \_\_\_\_\_

185

Equilibrate O/N with buffer D, P182 (50 mM KCl) → see P183 for conductivity of col effluent  
Load ~ 60 ml dialysate (P183) at 0.67 ml/min  
= 2 col vol/hr (as done on P11 for rTag)  
(1 min/min)

wash ~ 1 col vol 0.67 ml/min

for gradient want to make it fairly flat for first try  
of TPI on Heparin.

Gradient:

50-700 mM KCl (= 0-35% pump B since  
E is 2 M KCl)

20 col vol = 400 ml, 4 ml/hr (so 100 fractions total)

run at 2 col vol/hr

so need 10 hours for whole gradient

rTag comes off Heparin in 400 mM KCl (see P 46)

so might see TPI ~ 6 hr post start ~ late afternoon  
if TPI same as Tag

(loading done ~ 10:25 AM wash 30 min (= 1 col vol)  
gradient start ~ 11 AM

100 5 BANK 1

.00 CONC AB  
.00 CONC AB  
.00 ML/MIN  
.00 PORT.SET  
.00 PORT.SET  
.00 VALUE.POS  
.00 VALUE.POS  
.00 CONC AB  
.00 ML/MIN

To Page N . \_\_\_\_\_

ed & Und rstood by m ,

reana Polarp

Date

5/1/95

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Date

4-20-95

Page No. 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27

$R_{xu \min}$  48

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|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|-----|

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| 72 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 | 72 | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 93 | 94 | 95 | 96 | 97 | 98 | 99 | 100 |
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*[Faint handwritten notes at the bottom of the page]*

2000  
1999

7001  
12001

*[Handwritten signature]*

7/1/53 ( # 31 )  
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(#52)

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2006-24-32 p173  $\frac{1}{1/2000}$  (# 33, 34)

Analysis PIP3  $\frac{1}{1.2400}$  (735, 36) To Page No. \_\_\_\_\_

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| Page 2 of 2 | 5 |  | 4.7.5T |
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11/95 Recorded by 10/1/95

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Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

From Page No. \_\_\_\_\_

|    |    |         | pmol | u/pl  | average<br>u/pl | fraction                      | total<br>units |
|----|----|---------|------|-------|-----------------|-------------------------------|----------------|
| 38 | 1  | 321.00  | 12   |       | 1.9             |                               |                |
| 39 | 2  | 526.00  | 20   | 3.0   | 3.0             |                               |                |
| 40 | 3  | 1566.00 | 60   | 9.03  | 9.9 ave         |                               |                |
|    | 4  | 928.00  |      | 10.7  |                 | 4ml                           | 39600          |
|    | 5  | 513.00  |      |       |                 |                               |                |
|    | 6  | 326.00  |      |       |                 |                               |                |
|    | 7  | 3904.00 |      | 22.52 |                 |                               |                |
|    | 8  | 1849.00 |      | 21.35 | 21.9 ave        |                               |                |
| 41 | 9  | 1346.00 |      |       |                 | 4ml                           | 87600          |
|    | 10 | 792.00  |      |       |                 |                               |                |
| 42 | 11 | 5730.00 |      | 33    |                 |                               |                |
|    | 12 | 3486.00 |      | 40    | 40.5            | 4ml                           | 162000         |
|    | 13 | 1668.00 |      | 37    |                 |                               |                |
|    | 14 | 1117.00 |      | 51    |                 |                               |                |
| 43 | 15 | 5064.00 |      | 23    |                 |                               |                |
|    | 16 | 3156.00 |      | 36    | 41.3            | 2.68ml                        | 110684         |
|    | 17 | 1890.00 |      | 45    |                 |                               |                |
|    | 18 | 1239.00 |      | 57    |                 |                               |                |
| 44 | 19 | 6029.00 |      | 34    |                 |                               |                |
|    | 20 | 3974.00 |      | 43.8  | 43.8            | 2.68                          | 117384         |
|    | 21 | 2233.00 |      | 51    |                 |                               |                |
|    | 22 | 969.00  |      | 44.7  |                 |                               |                |
| 45 | 23 | 4489.00 |      | 25.5  |                 |                               |                |
|    | 24 | 2775.00 |      | 31    | 35.6            | 2.68                          | 95408          |
|    | 25 | 1960.00 |      | 41    |                 |                               |                |
|    | 26 | 858.00  |      | 39    |                 |                               |                |
| 46 | 27 | 2156.00 |      | 12.4  | 12.3            | 2.68                          | 32964          |
|    | 28 | 1056.00 |      | 12.1  |                 |                               |                |
|    | 29 | 843.00  |      |       |                 |                               |                |
|    | 30 | 364.00  |      |       |                 |                               |                |
| 47 | 31 | 847.00  |      |       | 4.9             |                               |                |
| 48 | 32 | 465.00  |      |       | 2.7             |                               | 13000          |
| 49 | 33 | 4246.00 |      |       |                 |                               |                |
| 50 | 34 | 2441.00 | 93.9 | 14.0  |                 | 54ml                          | 756000         |
| 51 | 35 | 3795.00 |      |       |                 | 60ml                          |                |
| 52 | 36 | 2266.00 |      | 13.9  |                 | off dialysis                  | 751000         |
| 53 | 37 | 165.00  |      |       |                 | 33 100% recover from dialysis |                |

32.5 CFU/pmol

(accidentally omitted  
47 pl as incorrect  
pmol fraction)

Witnessed &amp; Understood by m ,

Deena R. Ship

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To Page 1

Page N \_\_\_\_\_

|                | <u>units</u> | <u>% recovery</u> |
|----------------|--------------|-------------------|
| C'/PEI         | 1,210,000    | 100%              |
| monium sulfate | 1,280,000    | 100               |
| phacryl 200    | 859,000      | 71                |
| sepharose      | 756,000      | 62                |
| alysis         | 751,000      | 62                |
| arin AF        | 666,000      | 55                |

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d &amp; Understood by me,

Sandra Polansky

Date

5/1/95

Invented by

Recorded by

Date

4-21-95

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Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

From Page No. \_\_\_\_\_

Tet stock

grow  $\lambda$ pl, sp6 plasmid  
in host lacking TFI sp6 plas  
but containing Tet resistance  
plasmid with  $\lambda$  promoter and  
sp6 gene

LB 50ml  
Tet 30  $\mu$ g/ml  
add start of cells  $\rightarrow$  30°C shaking O/N

got no growth O/N! 4-24  
 $\rightarrow$  no it just grew  
slow - had cells 36 hr

Repeat with m. longi Tet and  
at only 15  $\mu$ g/ml  
after 36 hr grow O/N at 30°C  
(including stock used above that &  
should dissolve "crystalline" TC in E

made fresh ~~Tet~~ TC stock  
^ TC H<sub>2</sub>O in H<sub>2</sub>O first

13.9 mg Sigma No. T3258 Tetracycline  
a little water  
(it still doesn't go into solution)

100% ETOH (good stuff from Corning)  
up to 27 ml  
= 5 mg/ml stock in foil, -20°C

inoculate 1 ml of O/N #5 into 50 ml  
in circle grow + 5  $\mu$ g/ml of fresh TC stock  
shake at 30°C 30  $\mu$ g/ml

start 8:30 stop 3 PM got 0.22 mg cells  
so add 0.88 ml Taget buffer (P167,3) for 0.2  $\mu$ g/ml

Witnessed & Understood by me,

Deena a Polay

Date

5/1/95

Invented by

Recorded by

Date

4-23-95

To Page N

# Regeneration of columns

Project No. \_\_\_\_\_

Exhibit 76

Appl. No. 09/558,421

Book No. \_\_\_\_\_

191

e No. \_\_\_\_\_

Blue sepharose

2 col vol 6M Guanidinium HCl  
5 col vol H<sub>2</sub>O (immediately)  
2 col vol 20% EtOH for storage

Heparin Af

2 col vol 4M urea  
2 col vol H<sub>2</sub>O  
2 col vol 20% EtOH

(0.3 - 0.5 M NaOH recommended)

spharose S200

1/2 - 1 col vol 0.4M NaOH

contact with col =  $\geq 1 \text{ hr} \leq 2 \text{ hr}$

H<sub>2</sub>O 2 col vol

20% EtOH for storage

run 0.4M NaOH at 2 ml/min  
for 45 min (= 1/2 col vol)

(start 10:20 am) H<sub>2</sub>O for 3 hr at 2 ml/min  
= 2 col vol and NaOH only in  
contact with column for  
45 min + 90 min

20% EtOH 3 hr 0.2 ml/min O/K

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Date

Invented by

Date

revised by

5/1/95

Recorded by

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Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

SDS gel for TFI prep

192

From Page No. \_\_\_\_\_

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

DH10BRL

Fr I P190

Fr I' (75°C, 30) P190

3λ

30λ

TFI fr I 2.45 u/l (P190)

3λ

fr I' 2.45 u/l (P190)

30λ

AmS04 resuspended

0.5

362 u/l

5200 u/l

3.5

Blue pool Fr 2432

12.5

14 u/l (P17P)

Heparm Fr #

39

3 u/l

5

40

9.9

5

41

21.9

5

42

40.5

5

43

41.3

5

44

43.7

5

45

35.6

5

46

12.3

5

47

4.9

5

TFI experiment 1 u/l

30

cut TF31010A-502

2X sample buffer

30

H<sub>2</sub>O

27-27-3027 17.525

load 15 u/l MW standards

CTI cut 10064-012

run at ~29 mA

started 9:15 AM

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Witnessed & Understood by me,

Deena R Polamp

Date

5/1/95

Invented by

R c r d b y

Date

4-25-95

47 cm Heparin AF column (80 ml)

Project N

Exhibit 78

Block N

Appl. No. 09/558,421

1

Page No.

proved a 1.5 cm x 47 cm (80 ml) column  
to try to separate the 2 peaks on P186-187, 9  
flow rate is 0.204 ml/min by gravity.

gradient will be 50 mM - 400 mM  
and 10 col vol = ~~1000 ml~~ 1600 ml  
so gradient 1/2 as steep as P185, 9: 20 ml col

pool fr 40-43 (14.7 ml total)  
of Heparin (see P185-192, 9)

Dialyze ON against 1 L buffer D

(frms are ~ 300 mM KCl  
so expect ~ 4.2 mM + 50 mM in buffer D)

start gradient ~ 9:30 AM

gradient is

1600 ml (20 col vol)

50 mM - 400 mM KCl (was 50 mM - 700 mM)

2 ml in 5 ml/min, so <sup>13.3</sup> 13.3 hr for gradient

4.5 hr/min / frn = 9 ml / frn (20 frms total)

note 1.5 ml/min gave only 0.2 mPa (column  
is definitely running with backpressure) but  
2 ml/min still only ~ 0.2 mPa so will  
use 2 ml/min  $\Rightarrow$  1.5 col vol/hr  
used 2 col vol/hr for 20 ml col P185, 9

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4-27-95

From Page No. \_\_\_\_\_

expect protein to start coming off at  $\sim 65\%$  of the  
gradient is  $\sim 1040 \text{ ml} = 8.7 \text{ hrs.}$   
or  $\sim 6:30 \text{ pm}$

since pol started coming off at  $13\% \text{ } \phi + 50 \text{ mm}$   
 $= 410 \text{ mm}$

### Comparison of 80 and 20 ml columns

|               |                             |                             |
|---------------|-----------------------------|-----------------------------|
| col vol       | 20 ml                       | 80 ml                       |
| col height    | 11 cm                       | 47 cm                       |
| gradient vol  | 20 col vol                  | 20 col vol                  |
| gradient stop | <u>35 mm KEE</u><br>col vol | <u>20 mm KEE</u><br>col vol |
| flow rate     | 2 col vol/hr                | 1.5 col vol/hr.             |

Therefore the new col is  $4\times$  longer has  $0.75\times$   
flatter gradient and is  $0.75\times$  slower flow rate  
so hope to get better separation of 2 peaks see  
on p 186-187, 9

THOD 5 BANK 2

1.00 CONC XB C  
1.00 CONC XB C  
.00 ML/MIN 2.  
.00 PORT.SET 3  
.00 PORT.SET 6  
.00 VALVE.POS 1  
.00 VALVE.POS 2  
0.0 CONC XB 20  
0.0 ML/MIN 0.1

Witness d &amp; Und rsto d by me,

Deeann Polarp

Date

5/1/95

Invented by

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Date

4-27-95